THE

BOTANICAL MAGAZINE

PUBLISHED

BY

THE BOTANICAL SOCIETY OF JAPAN

Volume 73 – 4

Nos. 859–870

TOKYO

1960 – 61

植　物　学　雑　誌

第 73 巻 859—870 号

日本植物学会発行

東　京

1960

AUTHOR INDEX

Articles

Short Communications

Miscellaneous Notes

索　　引

(アイウエオ順)

論　説

短　報

雑　録

Cytological and Morphological Studies on the Gametophytes of Ferns XIII The Permeability of the Wall Cell of Antheridium to Urea and Glycerol*

by Isami IGURA**

Received March 2, 1959

In the study on the growth and morphogenesis of fern-prothallium, Reuter[1]) examined the permeability of prothallial cell. Igura[2]) also has carried out the cyto-physiological and further the microchemical studies concering the plasmolysis form, the osmotic value, the permeability to urea and glycerol, the iodine reaction of starch-grain and the nucleic acid in the chloroplast, and TTC reaction with the prothallial cell in the course of the prothallium. He[3]) has also investigated the permeability of the prothallial cell to urea and glycerol by means of plasmolysis and has pointed out that the meristematic portion (apical part) of the prothallium has the glycerol-type permeability, whereas the protonema portion of the prothallium has the urea-type. Hereupon, to determine the permeability-ratio between urea and glycerol is significant for clarifying the characteristics in permeability of the wall cell of antheridium.[1,3,4,5,6]) No investigation has been carried out from this point of view up to now. Hence, the present study was undertaken for the purpose of making this point clear and of understanding the dehiscence mechanism of antheridium.

Materials and Methods

Antheridia of the following species were used as materials: *Athyrium melanolepis* Christ, *Athyrium rupestre* Kodama, and *Leptogramma totta* J. Smith. Of these species the last one was mainly used in this experiment.

First, behaviors of plasm of the wall cells were examined when they were plasmolysed with aqueous solution of urea or glycerol (1.0, 1.2M). And then, the plasmolysis time was determined with the aqueous solution of glucose (1.0, 1.2, 1.4M) in the same way as Reuter[1]) in order to know the permeability to water.

For the determination of the permeability-ratio between urea and glycerol, the deplasmolysis time of wall cell of the antheridium in the aqueous solution of urea and glycerol (1.0, 1.2, 1.6M) was measured. The permeability coefficient (μ) was calculated by the formula of Tröndle,[7,8]) $\mu=1-C/C'$, where C is the concentration of aqueous solution of sucrose which is given by the incipient plasmolysis; C′ is that of urea or glycerol.

Results

The plasmolysis form and behavior of plasm in the wall cell.

The plasmolysis form of the wall cell represented usually convex type, while the prothallial cell showed the concave one. These bearings did not change before and after the extrusion of spermatids out of the antheridium.

The plasmolysis began to arise from a corner of the outer membrane of the

* Supported by the grant from the Fundamental Scientific Research of the Ministry of Education for "Associated study on the Pteridophyta".

** Biological Institute, Faculty of Education, Yamagata University, Yamagata, Japan.

wall cell (Figs. 1 A, A′). The protoplast including cellular entities such as, several chloroplasts, and granules which were stained with the 1% aqueous solution of janus green and hence thought to be chondriosomes, approached toward the inner membrane gradually. Finally, it adhered to a slightly lower portion of the inner membrane of the ring cell, displaying the spheric state of perfect plasmolysis (Figs. 1 B, B′). In the basal ring cell the behavior of plasm is the same as the case of the other part of the ring cell, except that the plasm which had reached to the spheric state was in contact with slightly upper portion of the inner membrane of the basal

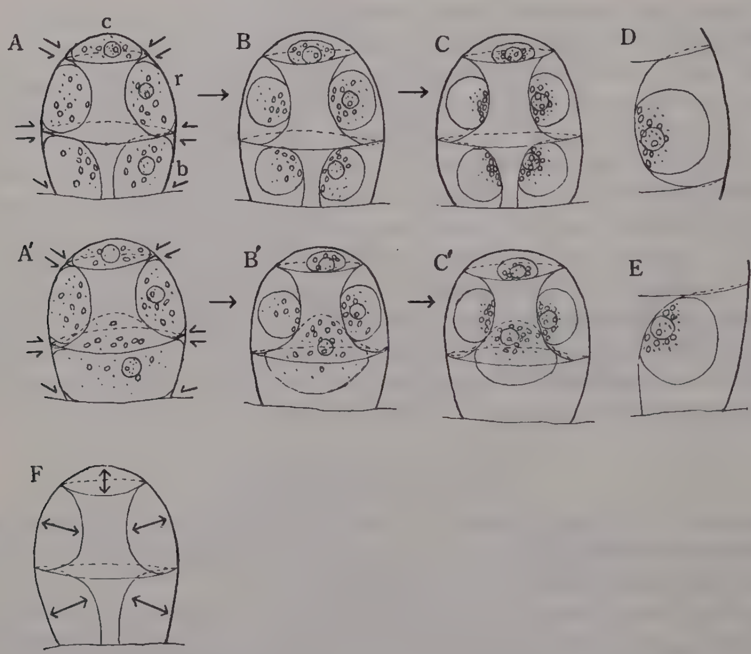


Fig. 1. A somewhat schematical representation of the plasmolysis form, and the behavior of plasm and cellular entities in 1.0M glucose solution in the wall cells of antheridium of *Leptogramma totta*. (×270). A′ is an antheridium whose lower membrane of the central cavity does not reach to the basal membrane of basal cell, so that the basal ring cell is not formed.

→: the locus of plasmolysis.

→: indicates the progressive change of the form of plasm and the situations of cellular entities.

c, cap cell; r, ring cell; b, basal ring cell. A, A′, incipient plasmolysis; C, C′, "Plasmasystrophe"; D, E, "Plasmasystrophe" in the ring cell and the basal ring cell respectively; F, Directions of the polarity shown by arrows.

ring cell (Figs. 1 B, B′). Thus the "Plasmasystrophe"[3]) occurred generally after about 3 hours (Figs. 1 C, C′, D, E). From these facts the polarity concerning plasmolysis is considered to exist even in a single wall cell, either the ring cell or the basal ring cell of antheridium. And the polarity seems to take the direction from outer-side toward inner-side as was shown in Fig. 1 F.

The plasmolysis time and deplasmolysis time.

The result of experiment of plasmolysis time with the aqueous solution of glucose is given in Table 1.

Table 1. The plasmolysis time (minutes) of the wall cell of antheridium with the aqueous solution of glucose, compared with that of the prothallial cell of *Leptogramma totta*. (Room temperature, 19°; water temperature, 18.5°)

M	Wall cell		Prothallial cell				
	Ring cell	Basal ring cell	*I	II	III	IV	V
1.0	6–7	7–10	10–13	10–15	20–25	20–25	25–35
1.2	6–8	7– 8	8–10	10–15	15–20	20–25	25–35
1.4	4–5	4– 6	5–10	5–10	10–15	15–20	20–30

* Different regions of the prothallium.[3] (VI region is omitted).

Results of the experiments of deplasmolysis time with the aqueous solutions of urea and glycerol are shown in Table 2.

Table 2. The deplasmolysis time (minutes) of the wall cell of antheridium with the aqueous solutions of urea and glycerol compared with that of the prothallial cell of *Leptogramma totta*. (Temperatures are the same as Table 1)

Agent		Deplasmolysis time							Plasmolysis time						
		Wall cell		Prothallial cell					Wall cell		Prothallial cell				
	M	Ring cell	Basal ring cell	*I	II	III	IV	V	Ring cell	Basal ring cell	*I	II	III	IV	V
Urea	1.0	180–190	185–195	200–215	190–210	185–200	175–190	165–200	5–7	7–10	10–18	10–18	20–30	35–45	40–50
	1.2	190–200	190–210	215–225	195–215	195–215	190–200	175–190	4–6	6– 8	10–15	10–15	20–30	30–40	40–50
	1.6	200–215	200–215	235–245	235–245	200–220	200–220	165–180	4–5	5– 7	7–10	7–10	15–25	25–35	35–45
Glycerol	1.0	150–160	145–160	220–230	195–215	180–190	140–145	125–140	6–9	8–10	20–25	25–30	45–55	55–67	70–90
	1.2	160–185	150–170	230–240	200–220	180–195	150–160	130–150	6–8	6– 9	15–20	20–30	40–45	55–65	60–80
	1.6	170–190	170–185	250–260	240–250	200–215	180–195	140–160	5–7	5– 8	10–15	15–25	30–40	45–55	50–60

* The same as Table 1.

Reuter[1]) has presumed that water-permeability based on the rate of plasmolysis in the aqueous solution of glucose and she assumed that the time necessary for the cell to reach to the state of perfect plasmolysis seemed to be in correlation with the viscosity of the plasm. According to the results of her experiments with 1.0M aqueous solution of glucose, longer plasmolysis time was shown in the younger cell. And then, she concluded that this phenomenon may be due to higher cohesiveness between plasm and membrane and smaller permeability to water.

From the results obtained in Table 1 of this paper it is shown that the plasmolysis time of the wall cell of antheridium was shorter than that of the prothallial cell, when they were plasmolysed with 1.0M (1.2, 1.4M also) aqueous solution of glucose (the similar tendency in plasmolysis time with the aqueous solutions of urea and glycerol was observed as was given in Table 2). From this fact it may be considered that the cohesiveness between plasm and membrane of the wall cells should be slight and the water-permeability of these cells higher. In wall cells of the antheridium, the plasmolysis time of the ring cell is a little shorter than that of the basal ring cell. This phenomenon may be due to the fact that the cohesiveness between plasm and membrane of the ring cell is a little low and also the permeability to water is high.

Results of Table 2 indicate that the deplasmolysis time of the wall cell is shorter on the average than that of the prothallial cell in either aqueous solution of urea or glycerol and the deplasmolysis time of the wall cell in the aqueous solution of glycerol is generally shorter than that in the aqueous solution of urea. Considering from these results, it is clear that the permeability of the wall cell to both urea and glycerol is higher than that of the prothallial cell, and the permeability of the basal ring cell to glycerol is higher than that of the ring cell. Concerning the permeability-ratio between urea and glycerol, the wall cells of both ring cell and basal ring cell of the antheridium are more permeable to glycerol than to urea. Accordingly type of permeability of wall cells belongs to glycerol-type. This type of permeability is generally found only in the younger cells of meristematic region of the prothallium.[3]) This fact is considered to be noteworthy.

According to the results of the preceding study,[11]) the osmotic values corresponding to the suction force of the wall cell were 0.98M aqueous solution of sucrose and 0.92M of urea respectively. In the present investigation, meanwhile, the osmotic value corresponding to the suction force was measured to be 0.96M aqueous solution of glycerol, using the same method as in the case of the preceding study. Therefore, the permeability coefficients (μ) of urea and glycerol were -0.07 and -0.02 respectively. Thus the reason why the permeability-ratio of sucrose to these substances becomes to negative value seems to be due to the fact that both urea and glycerol are more permeable than sucrose to the wall cells. From this fact, it may be concluded that the permeability coefficient of glycerol is larger than that of urea for the wall cell of antheridium.

Discussion and Conclusion

The plasmolysis type of the wall cell was usually convex type. Furthermore, the contracted plasm by plasmolysis located itself adhering to the inner membrane of the wall cell (membrane of the central cavity). The writer[3]) reported previously the phenomenon, " Plasmasystrophe " occurred in the prothallial cell. The similar " Plasmasystrophe " was observed in the wall cell at the side of inner membrane. This fact implies that the physico-chemical nature of the protoplast at the inner- and outer-portions of the wall cell is not uniformly constructed even in a single wall cell. This caused the presence of physiological polarity within a cell, taking the direction from outside toward inside. Therefore, swelling of wall cells of the antheridium runs along the direction of this polarity upon the dehiscence of antheridium. This mechanism appears to be advantageous for the dehiscence of antheridium, and consequently the extrusion of spermatids.

The wall cell is more permeable to glycerol than to urea. This phenomenon is convincible by the fact that the permeability coefficient of glycerol is larger than that of urea. Mückschitz[5]) examined the permeability of the floral leaf-cell in *Crocus*

vernus, *Tulipa Gesneriana*, etc. to urea and glycerol. According to him, the epidermal cell in the budding stage of these plants revealed a higher value in permeability, while the permeability to urea and glycerol of the matured or old flower became gradually lower. According to Url,[6,12] the permeability of the epidermal cell of young basal zone of the peduncle of *Taraxacum officinale* exhibited the glycerol-type, whereas that of old apical zone did the urea-type. These facts may draw the conclusion that the active young cell reveals higher permeability and the glycerol-type of permeability. The permeability type of the wall cell of antheridium was glycerol-type which was generally seen in cells of meristematic region of the prothallium, differing from the other region of the prothallium where the urea-type is usually seen. From these facts it may be concluded that the glycerol-type of permeability is a characteristic of the wall cell which is in high activity. Thus, the evidence that the wall cell of antheridium has the glycerol-type of permeability seems to be significant for the understanding of mechanism of antheridium.

Summary

Concerning the permeability of the wall cell, i.e. the ring cell and the basal ring cell of antheridium of *Leptogramma totta* J. Smith mainly to urea and glycerol, the following facts were cleared.

1. As to the plasmolysis form and the behavior of the wall cell of antheridium, it was ascertained that the plasmolysis form was convex-type, the plasmolysis began to arise at the corner of outer plasm, and the "Plasmasystrophe" was observed at the inner part of the wall cell. These facts indicate the existence of physiological polarity even in a single wall cell in the direction from outside toward inside.

2. The type of permeability of the wall cell belongs to the glycerol-type which was found in the meristematic region of the prothallium.

3. It may be considered that the glycerol-type of permeability is a characteristic of the highly acting cell and that the activity of the wall cell of antheridium is also high. This fact may be advantageous for the dehiscence of antheridium.

The writer expresses his cordial gratitude to Prof. A. Yuasa, University of Tokyo, and Prof. T. Miwa, Tokyo University of Education, for their criticisms and suggestions and for their revising the manuscript.

References

1) Reuter, L., Protoplasma **42**: 1 (1953). 2) Igura, I., Lecture delivered on the meeting of "Pteridological Society of Japan" at Kyoto University, October 23rd, 1958 (Unpublished yet). 3) ——, Bot. Mag. Tokyo **67**: 119, 184, 208, 289 (1955). 4) Bogen, H., Planta **38**: 65 (1950). 5) Mückschitz, G., Protoplasma **40**: 348 (1951). 6) Url, W., ibid. **40**: 475 (1951). 7) Tröndle, A., Ber. d. Deut. Bot. Gesells. **27**: 71 (1909). 8) ——, Jahrb. f. wiss. Bot. **48**: 171 (1910). 9) Hiraoka, T., Cytologia **17**: 191 (1952). 10) Nakazawa, S., Bull. Yamagata Univ. (Nat. Sci.) **2**: 225 (1953). 11) Igura, I., Bot. Mag. Tokyo **72**: 231 (1959). 12) Reuter, L., Protoplasmatologia **XI** (**2**) (1955).

摘　　要

シダ類の配偶体に関する細胞学的ならびに形態学的研究 VIII. 造精器壁細胞の尿素およびグリセロルに対する透過性

伊　倉　伊　三　美

ミゾシダ (*Leptogramma totta* J. Smith) を主な材料として用いた外，シダ類2種を材料とし，それら前葉体上に発育した造精器の壁細胞，すなわち主として環細胞，底部環細胞の尿素及びグリセロルに対する透過性についてしらべた結果，次のことがらがわかつた．

1. 壁細胞質はつねに凸型原形質分離をなし，原形質分離個所は壁細胞の外膜の角にある．そして，ついにその内膜に接して "Plasmasystrophe" の現象がおこる．このことは，単一の壁細胞にも生理学的極性の存在することを意味し，極性方向は外から内に向う．

2. 尿素とグリセロルの透過比から得た壁細胞の透過性の型はグリセロル型である．

3. グリセロル型であることは activity の高い，若い細胞に特徴的の型と考えられ，壁細胞は高い activity をもつものと推定され，このことは造精器裂開に都合のよいことであると考えられる．(山形大学教育学部生物学教室)

The Influence of Nutrients on the Growth of Plant Populations under Different Densities
Relations of Plant Communities to Edaphic Factors with Special Reference to Mineral Nutrition III

by Yasuhiko TEZUKA*

Received March 25, 1959

Since the classical work by Clements *et al.*[1]), many investigations have been made on the effects of density on the growth of plant populations[2,3,4,5,6,7]). Notable among them are the recent investigations by Kira *et al.*[4,5]), who have derived an empirical formula from their experimental results. Now it is a well-known fact that the density effect can be broadly seen in crop plantations as well as herbaceous or arboreous communities in the natural field. However, the mechanism which is working behind and representing the phenomena has scarcely been pursued so far. As Clements already stated, the mechanism should be looked for in the fact that plants within a population compete with each other for several factors, i.e. light, water and mineral nutrients, inevitable for their growth. And, the effects of the environmental factors are almost always so integrated that careful parallel analyses are needed for the proper evaluation of the role of each factor.

The present investigation was undertaken to clarify how mineral nutrients influence the growth of plant populations under different densities. Answering this problem, two series of experiments were carried out; the one was a preliminary experiment on the density effect of plant populations under water culture conditions and the other of artificial buckwheat communities under natural field conditions. The latter was carried out in co-operation with Dr. Iwaki, Mr. Kuroiwa and Dr. Midorikawa. Analyzing dry matter production of the buckwheat communities, Iwaki[7]) has succeeded in clarifying the significance of light factor for the growth of communities with different densities. Micro-climatological aspect and distribution of carbon dioxide in the communities have already been reported respectively by Kuroiwa and Monsi[8]) and Midorikawa[9]).

A. An experiment of density effect under water culture conditions

As experimental material *Brassica cernua* was used. After ten days from germination on well-boiled saw-dust, seedlings, whose mean dry weight was 1.8 mg., were transplanted to culture solutions of various concentrations. As water culture vessels were used Petri-dishes, which were 20 cm. in diameter and contained 700 ml. of solution, covered with black paper to prevent algae from growing in solutions. The nutritional levels were prepared by simple dilution at the concentrations of 1/1, 1/10 and 1/100 of normal solution, of which constitution was the same as that prescribed in a previous paper[10]), and at each nutrient level three plant densities, 5, 17 and 41 plants per vessel, were settled. Accordingly there came 9 sorts of different combination of nutritional levels and plant densities, being designated as 1/1–5, 1/10–17, 1/100–41, etc. These culture vessels were held in a frame outdoors for 30 days, from

* Department of Biology, Faculty of Science, Tokyo Metropolitan University, Tokyo, Japan.

October 25 to November 24, 1954. Throughout the culture period the solutions were not renewed, only with adding distilled water to keep the water level in the vessel constantly at 2 cm. below the wooden lid supporting the plants. After 30 days cultivation, the plants were harvested to measure their dry weights, and the amounts of principal nutrients remaining in the solutions were determined.

The plants of 1/100–41 series soon stopped their growth and then the plants of other series ceased to grow in the following sequence; 1/100–17, 1/100–5, 1/10–41, 1/10–17, 1/10–5 and 1/1–41. Only the plants of 1/1–5 and 1/1–17 series continued their growth up to the end of the cultivation. The plants which stopped their growth showed strikingly the visual symptom of nitrogen deficiency, i.e. pale-yellowish, sickly color of leaves[10]. The appearance of the plant populations of 30-days cultivation is seen in Fig. 1. Dry weight of individuals and that of the populations are illustrated in Fig. 2. From the results the following can be observed.

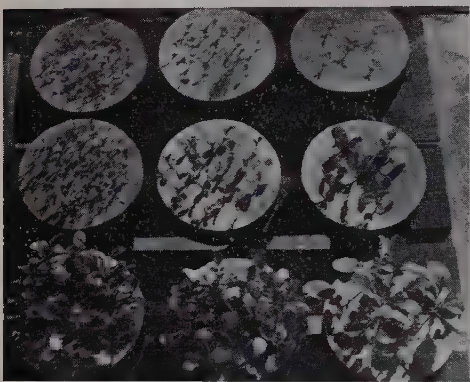


Fig. 1. A photograph showing the appearance of the *Brassica* plants grown under different densities and nutritional levels. *Upper group*: 1/100 conc. *Middle group*: 1/10 conc. *Lower group*: 1/1 conc. In every group, from left to right; 41, 17 and 5 plants per vessel.

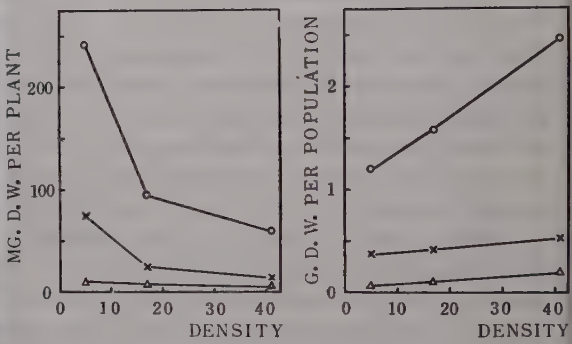


Fig. 2. Dry weights of individual (left) and population (right) of *Brassica* plants after 30-days cultivation. —○—: 1/1 conc., —×—: 1/10 conc., —△—: 1/100 conc.

At the same population density, maximum growth in individual as well as in whole population rose in accordance with nutrient supply increase, and at the same nutrient level, the higher the population density, the smaller became the maximum growth of individuals. The maximum total dry weight of populations, however, was at 1/10 and 1/100 nutrient level respectively a fixed value independently of the population density, in consideration of the difference in total weight of planted seedlings. At 1/1 nutrient level considerable difference was observed among dry weights of the populations of different densities: this might be due to the fact that the maximum plant growth was not reached at the 1/1–5 and 1/1–17 series by the end of the cultivation. A definite maximum would also be expected for the final dry weight of populations with different densities at this high nutrient level, because the maximum growth is usually not determined by ion concentration itself but by the whole available amount of ions, as reported in previous papers[10,11].

In addition to such differences in dry weight growth, it is also interesting that the ratio between leaf, stem and root changes strikingly under the varying conditions

(Table 1). The higher the nutritional level and the smaller the plant density, the larger becomes the distribution of dry matter to leaves. This trend may be due to

Table 1. The ratio of leaf, stem and root of water-cultured *Brassica cernua*, expressed by percentage of dry weight, and C/F ratio (the ratio of non-photosynthetic system to photosynthetic system).

Group	Series											
	5				17				41			
	Leaf	Stem	Root	C/F	Leaf	Stem	Root	C/F	Leaf	Stem	Root	C/F
1/1	81	3	16	0.23	76	7	17	0.32	75	8	17	0.33
1/10	73	5	22	0.37	60	12	28	0.67	54	15	31	0.85
1/100	46	9	45	1.17	47	13	40	1.13	44	18	38	1.27

the fact that a large amount of mineral nutrients, especially of nitrogen, is generally required for the normal development of leaves, and as a whole it coincides with Iwaki's results in buckwheat concerning C/F ratio (the ratio of non-photosynthetic system to photosynthetic system). On the other hand, chemical analyses of the nutrient solutions at the end of cultivation clearly showed that all of nitrogen, phosphorus and calcium were exhausted in every series but 1/1–5.

From these results the observed density effect seems to be elucidated mainly as the competition among plant individuals for the nutrients, because other factors, including light factor, than mineral nutrients were kept relatively constant throughout the experiment, especially in case of the lower nutrient level. At the highest nutrient level some competition for light might occur among crowded plants at the later stage of cultivation. Moreover, such a result as that obtained is also seen in Knapp's theoretical treatment.

B. An experiment under field conditions

As for culture and sampling methods details have already been reported by Iwaki[7], so the procedure will be stated here only briefly. Seeds of buckwheat (*Fagopyrum esculentum*) were sown in a square disposition of 5 cm., 10 cm. and 20 cm. spacing (400, 100 and 25 plants/sq. m., resp.), in an experimental field of the Toride Upper Secondary School, Ibaraki Pref., on June 2, 1955. Prior to the sowing the field soil was fertilized with 15 g. of chemical manure per square meter, containing 10% total nitrogen, 7.0% soluble phosphate and 6.0% soluble potassium. The first sampling was made on June 11 and further samplings were done on every 7 days.

In order to clarify the relation of soil nutrient factors to the growth of the plant populations, the nutrient content of leaves, stems and roots, and the total amounts accumulated in the stands of each plot were determined.

Chemical analyses of total nitrogen, phosphorus and iron in the plant tissues were performed according to the methods described in a previous paper[10]. Potassium and calcium content were determined with a Perkin-Elmer-flamephotometer. Magnesium was determined as the difference between the sum total of Ca+Mg measured with EDTA reagent and the amount of Ca determined beforehand.

The growth of the buckwheat populations:—The growth of these buckwheat populations was discussed in detail in Iwaki's paper[7]. Accordingly, only main points will be summarized here. The difference of dry weights of the population or of

standing crop/sq. m. among the 5 cm., 10 cm. and 20 cm. plots became smaller and smaller with growing up of the plants, for example at the end of the experiment, on July 23, the values were 622 g., 544 g. and 490 g. (1:0.87:0.79) respectively. On the contrary, weights of individuals at that time were widely different among the plots, i.e. in the ratio of 1:4:13(cf. also Kira *et al.*[4,5])). Moreover, the interesting fact was the higher C/F ratio of the denser populations in the logarithmic phase of growth, e.g. on July 25, 1.64 in the 5 cm. plot against 1.11 in the 10 cm., 0.67 in the 20 cm. plot. These results coincide in general with those of the above mentioned water culture of *Brassica cernua*.

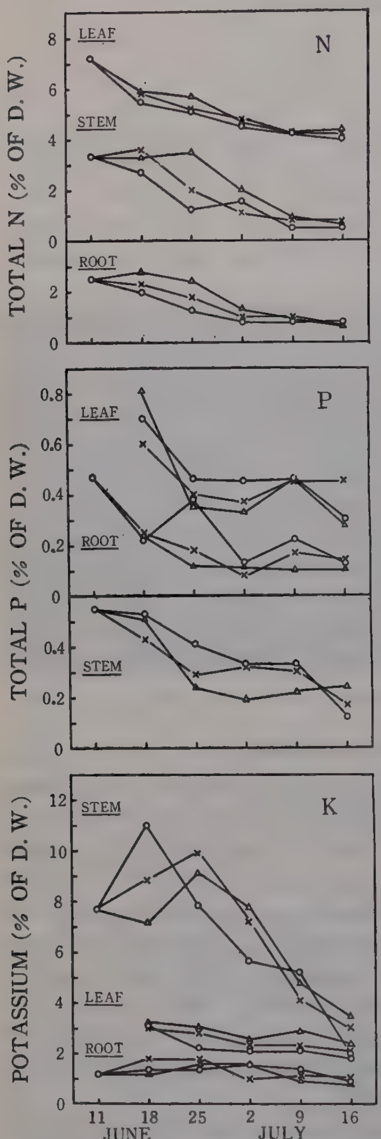


Fig. 3. Changes of concentration of total nitrogen, phosphorus and potassium at successive stages of growth in leaves, stems and roots of the buckwheat plants. —○—: the 5 cm. plot, —×—: the 10 cm. plot, —△—: the 20 cm. plot.

Further analytical discussion is necessary to elucidate the mechanism, which was working to make the different growth of the buckwheat populations with different densities. Concerning light factor and dry matter production of these populations a paper has already been submitted by Iwaki[7]). In that case he has succeeded in explanation of the growth difference by reconstruction of growth curves with photosynthetic and respiratory activities, C/F ratio, leaf area and light conditions in the communities, without special consideration of mineral nutrient factors. It is, however, generally very difficult in the field research definitely to clarify which factor can influence the growth of a plant or plant community decisively, because any single factor can not change itself under natural conditions without changing the other factors[13]). Consequently in the present paper, attention was focused particularly upon how much amount of nutrients was absorbed by plants under different density conditions, which might largely influence the plant growth. This phase of investigations may have practically considerable significance for the proper management of soil fertility.

Dynamics of mineral nutrients within the plant populations:—The changes of nitrogen, phosphorus and potassium concentrations in leaves, stems and roots at successive stages of growth in each plot are indicated in Fig. 3. Nitrogen concentration in each organ decreased with growth, and the denser the population, the rapider was the decrease just in the most vigorously growing period, so the lower nitrogen concentration was observed in the denser population, especially in roots. Phosphorus concentration also decreased generally with growth of each organ, but a trend of slight accumulation of phosphorus could be seen

in each organ of the 5 cm. plot, though the graphs showed considerable variability. This seems rather indirectly to indicate symptoms of nitrogen deficiency in the plants of the highest population (cf. 10). Potassium concentration maintained almost a constant level or decreased very slightly in leaves and roots throughout the experimental period, while its concentration in stem was as high as 8 to 10% in the early developmental stage, but rapidly decreased with plant growth up to the same level as in leaves. In the potassium concentration in leaves the lowest was observed at the highest population.

Fig. 4 illustrates the nutrient accumulation by the whole buckwheat stand (per sq. m.) in the 5 cm., 10 cm. and 20 cm. plots. These values were calculated from the

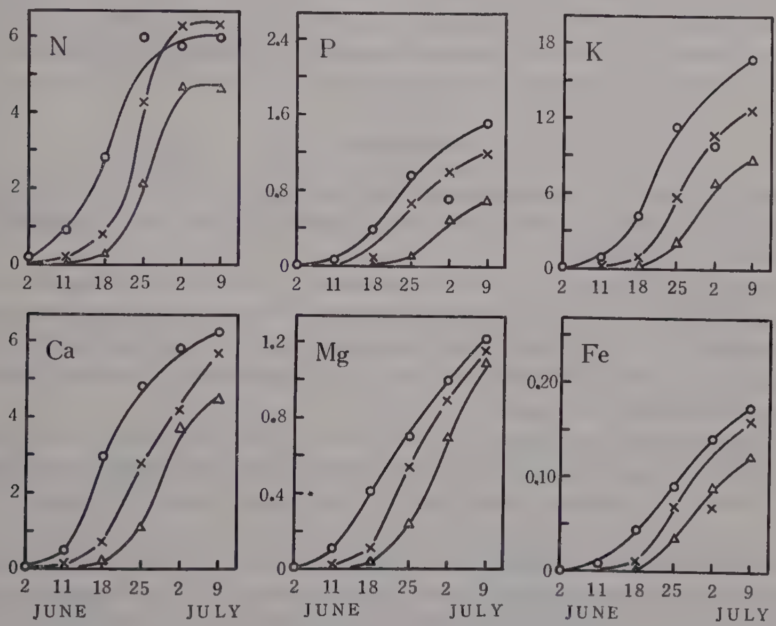


Fig. 4. Amounts of accumulated nutrients (g./m.² land area) in buckwheat stands of three kinds of spacing. —○—: the 5 cm. plot, —×—: the 10 cm. plot, —△—: the 20 cm. plot.

nutrient concentration and dry weight of each organ, as total sum of nutrients accumulated by plants. The amount of every nutrient accumulated in the stand increased of course in parallel with plant growth, and no limitation was observed in any element other than nitrogen, by July 9. As for nitrogen there was no conspicuous difference among the maximum amounts accumulated by the populations of different densities, though the amount absorbed in the 20 cm. plot was slightly lower than that in the other plots, because of low standing crop, or rather small leaf amount (in the 5 cm., plot 85.2 g., in the 10 cm. 92.5 g., and in the 20 cm. plot 61.2 g. /sq. m.), and it was noticeable that the highest accumulation was already obtained on July 2 in every plot.

From the facts that the difference in the concentration of nutrients among the populations was small and the limitation of nutrient, other than nitrogen, did not occur, the nutrient salts seemed hardly to be responsible for the growth difference among the stands with different densities, as we see in *Helianthus tuberosus* stands

(Hogetsu *et al.*[14]), in press), and so this might make possible to obtain good coincidence of Iwaki's calculated growth curves, which he constructed with consideration mainly photosynthetic and respiratory activities and light conditions, with the observed ones. In the case of calculation, however, he had also to take into consideration the large value of C/F ratio and low photosynthetic activity of leaves in the highest density plot of 5 cm. spacing. These features in the 5 cm. plot seems to be caused rather by weak nitrogen deficiency, as indicated by low nitrogen concentration (and also high phosphorus concentration) in each organ. Moreover, the accumulated nitrogen amount was 5–6 g./sq. m. and this was 3–4 times larger than the supplied nitrogen in fertilizer, though high nutrition supply would also be expected from the field soil. These results may suggest for the sake of complete elucidation of density effect the necessity of further precise investigation on the micro-rhizosphere of the individual plant and on the relation between nutrient supply and morphological and physiological changes of the plant grown with varying density.

Summary

1. Relations between nutrient supply and growth of plant populations with varying densities were studied in *Brassica cernua* water-cultured, and in buckwheat cultivated in the field (cf. Iwaki[7]).

2. Generally speaking, the larger the density, the lower becomes the maximum growth of individuals. So the maximum growth of the whole population has a tendency to converge to a fixed value under a given condition and, especially in the case of lower nutritional level, is limited by amount of nutrients available to plant growth, without interference of light factor.

3. In the buckwheat stands some limitation of accumulation was observed in nitrogen after its rapid absorption in the early stage of growth, but phosphorus, potassium, calcium, magnesium and iron did not indicate such limitation of accumulation.

4. The low nitrogen concentration in each organ of the buckwheat plants of the highest density with 5 cm. spacing seems to be a reason for low photosynthetic activity of the leaves and high C/F ratio (the ratio of non-photosynthetic to photosynthetic system): these two features generally cause the low growth rate of the plants in the overpopulated stand with depleted light conditions.

The author is indebted to Prof. K. Hogetsu and Prof. M. Monsi for their advice and criticism. A part of the present investigation was financed by a grant-in-aid from the Research Fund of the Ministry of Education.

References

1) Clements, F. E., Weaver, J. E. and Hanson, H. C., Plant Competition, Carnegie Inst. Washington (1929). 2) Hodgson, G. L. and Blackman, G. E., Journ. Exp. Bot. **7**: 147 (1956). 3) —— and ——, ibid. 8: 195 (1957). 4) Kira, T., Ogawa, H. and Sakazaki, N., Journ. Inst. Polytech. Osaka City Univ. **4**: 1 (1953). 5) ——, —— and ——, ibid. **5**: 1 (1954). 6) Satoo, T., Nakamura, K. and Senda, M., Bull. Tokyo Univ. Forests. **48**: 65 (1955). 7) Iwaki, H., Jap. Journ. Bot. **16**: 210 (1958). 8) Kuroiwa, S. and Monsi, M., Journ. Agr. Meteor. **12**: 41 (1956). 9) Midorikawa, B., Jap. Journ. Ecol. **7**: 72 (1957). 10) Tezuka, Y., Bot. Mag. Tokyo **71**: 181 (1958). 11) ——, ibid. **72**: 101 (1959). 12) Knapp, R., Experimentelle Soziologie der höheren Pflanzen, Stuttgart (1954). 13) Billings, W. D., Quart. Rev. Biol. **27**: 251 (1952). 14) Hogetsu, K., Oshima, Y., Midorikawa, B., Tezuka, Y., Sakamoto, M., Mototani, I. and Kimura, M., Jap. Journ. Bot. **17** (in press).

摘　　要

密度の異なる植物集団の生長におよぼす養分の影響
無機栄養からみた植物群落と土壌条件の関係 III

手　塚　泰　彦

栽植密度による個体あるいは群落の生長のちがいと養分要因の関係を明らかにするため，密度を変えたカラシナ (*Brassica cernua*) の水耕実験およびソバ (*Fagopyrum esculentum*) の圃場実験を行なつた．後者は岩城，門司，黒岩，翠川等との共同研究として行なわれた[7,8,9])．主な結果は次のようである．

カラシナの水耕実験： 濃度 1/1, 1/10, 1/100 の培養液[10,11]) 700 ml. を容れた，径 18 cm. のペトリ皿に，液を更新することなく 5, 17, 41 個体のカラシナのめばえを水耕した．同一密度では養分供給量が大きい程，個体及び群落全体の生長量は増大する．同一養分供給量では密度が大になるにつれて個体の最大生長量は減少し，群落の最大生長量は密度に関係なく大体一定になる．この現象は養分の欠乏する時にのみ見られ，養分要因に関するせり合が主な原因と考えられる．

ソバの圃場実験： 5, 10, 20 cm. 間隔に圃場に播種したソバの群落の最大生長量は密度に関係なく大体一定の値に達した．養分要因の作用を明らかにするために，群落内での養分の吸収量と，単位乾物量あたりの濃度を N, P, K, Ca, Mg および Fe について追求した結果，特に密な群落では生長の後期に窒素の欠乏が認められ，これが光要因と共に群落の最大生長量を制限していると推論された．(東京都立大学理学部生物学教室)

Effect of Water Economy on Plant Growth 2
An Analysis of Water Economy of Water-cultured Tobacco Plant*

by Tsumugu TOTSUKA** and Masami MONSI**

Received July 31, 1959

As to the problems of the water economy of plants, innumerable investigations have been made on transpiration, water absorption and transmission of plants. However, most of them are not enough comprehensive to build up a general scope of water economy as a whole, because they are mainly concerned separately with any of the components of the water economy. A real necessity in discussion of the water economy is the quantitative appreciation of interrelations of the water absorption, transmission, and transpiration of plants.

Montfort[1]) reported that water balance of plants is determined by the ratio of their transpiration T to their water absorption A at unit time interval; when $T \leqq A$, plants can maintain their turgidity and normal activity, while $T > A$, they will wilt sooner or later whether wilting symptoms are visual or not. Moreover, Huber[2]) proposed an equation concerning the water economy of plants, in which he had defined many logical components responsible for the water balance, though the water transmission in stem and the leaf water content were neglected.

The purpose of the present study was analytically to elucidate the quantitative relationship of the amount of active roots to that of transpiring leaves in the water economy of water-cultured tobacco plants based on the experimental data reported in a previous paper[3]), where the material and method were mentioned in detail.

1. Development of root and leaf area:

In discussion of water economy in plants, it is, above all, necessary to pursue the relation between the growth of root surface responsible for the water uptake and transpiring leaves. The variations with time in leaf area and in root fresh weight given in Table 1 demonstrate clearly the following facts of interest: The relative growth rate of leaf area[4]) of water-cultured tobacco plants in the 2, 4, 6 and 8 cm.-sets where the figures indicate the depth of nutrient solution surface from the top of culture pots, was 0.83, 0.82, 0.62 and 0.59, respectively, i.e., decreasing with depression of water level of pots for first 3 days after beginning the experiment. During next 4 days, however, the value was the greatest at the deepest set to be 0.63, 0.69, 0.71 and 0.86 in each set. At the starting of the experiment, the ratio of the submerged part to the aerial part in roots showed naturally steep gradient between the sets, but afterwards the submerged roots of lower water level's sets increased so rapidly that the ratio in all sets became almost the same value only after 3 days. Judging from these facts, it is likely that the aerial part of roots increases in accordance with the growth of submerged root, that is, the part of roots relating to the water transmission may be developed with increase of the water absorbing roots.

The relationship between fresh weight of the submerged roots and their surface area was investigated. Suppose the shape of a submerged root to be cylindrical, the equation indicating the relation of the root surface S to its fresh weight W can be

* Supported by the Grant in Aid of Scientific Research of the Ministry of Education.
** Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

Table 1. Effect of water level lowering on the increase of leaf area, and of fresh weights of aerial and submerged parts of roots. The dry weight of them was tabulated in a previous paper[3]). Experiments were started on April 14, 1959.

Date	Water level lowering	Leaf area	Root fresh weight			the ratio of s/t
		sq. cm.	total (t) mg.	aerial (a) mg.	submerged (s) mg.	(%)
Apr. 14	2 cm.	55.7	424.0	180	244.0	57.5
	4 cm.	52.0	450.3	360	90.3	20.0
	6 cm.	54.6	443.0	404	39.0	8.8
	8 cm.	57.0	462.6	448	14.6	3.2
Apr. 17	2 cm.	102	980	527	453	46.2
	4 cm.	95	904	487	417	46.1
	6 cm.	88	834	487	347	41.6
	8 cm.	91	1039	655	384	37.0
Apr. 21	2 cm.	223	2036	893	1143	56.1
	4 cm.	196	1986	663	1323	66.6
	6 cm.	181	2039	1006	1033	50.6
	8 cm.	151	1808	1095	713	59.4
Apr. 25	2 cm.	357	3676	1946	1730	47.0
	4 cm.	333	2393	780	1613	67.3
	6 cm.	302	2436	1053	1383	56.7
	8 cm.	280	2638	1233	1405	43.1

obtained as follows; $S=4W/\rho D$, where ρ shows the specific gravity of submerged roots, which was measured with pycnometer as 1.1 in average, and D is the diameter of submerged roots, i.e., 0.33 mm. in average measured at 2 cm. depth in the solution, changing slightly with plant development. Therefore, it might be concluded that the root surface is of direct proportion to the fresh weight, or the fresh submerged root of 1 mg. had about 10 sq. mm. root surface. Unfortunately, the computation of S was done here without any considerations of root hairs because of technical difficulties.

In order to clarify the relation between root growth and leaf area increase, the variation with time in the already mentioned active root/leaf area ratio (cf. (3)), i.e., the ratio of submerged part of roots in mg. fresh weight (C_w) to leaf area in sq. cm. ($\overline{F}$) was charted in Fig. 1 by using the figures shown in Table 1 in a previous paper[3]). The ratios varied scarcely with time in the 2 cm.-set, but extremely increased in the other sets for first 3 days after starting the experiment, until they had reached nearly the same value in all sets. The relation between C_w and $\overline{F}$ was illustrated with a regression line in Fig. 2, i.e., $\overline{F}=187.2\,C_w+12.9$, obtained by the least square method, while no special relation was demonstrated between the total fresh weight of roots and the leaf area. From the regression line and the above mentioned surface-weight relation in root, it follows that the amount of water transpired from the leaf surface of 1 sq. cm. under 1 cm. Hg saturation deficit can in average be offset by the water absorption through the root surface of 0.5 sq. cm. (5 mg. f. w. or 5.3 cm. long).

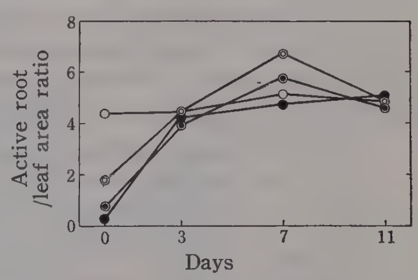


Fig. 1. Variation with time of the active root/leaf area ratio (mg. f. w./sq. cm.) at various water level's sets. (○ 2 cm., ◎ 4 cm., ⊙ 6 cm., ● 8 cm.-sets).

After all, the foregoing facts show exactly that the roots active in water absorption grow in proportion to leaf area increase after the active root/leaf area ratio recovered a normal value, and consequently may suggest that the ratio changes itself along with the water balance of the plant and can be maintained in a constant value under an environmental condition where a definite transpiration rate can be expected.

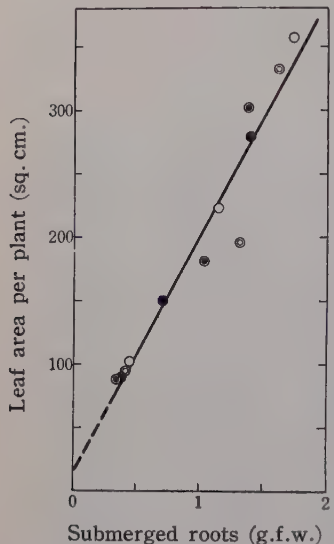


Fig. 2. Correlation between the leaf area and the active submerged root. The correlation coefficient is 0.97. Indications as in Fig. 1.

The relationship between the changes of the active root/leaf area ratio and the water deficiency of plant organs has already been discussed in a previous paper (see, Totsuka and Monsi 1959[3]), p. 370).

2. Leaf water index as a measure of a leaf water content:

The water content of a leaf is usually expressed in percentage of fresh or dry weight. But these values are variable ones even under normal leaf water conditions, as pointed out by Koketsu[5]). For example, the percentage on the dry weight basis can fluctuate with two variables, the amount of water and that of dry matter contained in unit leaf area, as in the following equation;

$$\text{Water content (\%) on dry weight basis} = \frac{(W_f - W_d)/\overline{F}}{W_d/\overline{F}} \times 100$$

where W_f, W_d and $\overline{F}$ indicate fresh and dry weight of leaves and their area, respectively.

For the sake of clarifying this, the results of some experiments will be shown briefly. The changes of water content on both bases were examined in two equally grown tobacco plants of about 1 g.d.w., water-cultured under the experimental conditions reported before[3]), 30 days after sowing. One of them was allowed to wilt by picking up the roots from culture solution for 3 hours and the other was kept in the normal conditions. As clearly shown in Table 2, in the wilted plant the water amount in unit leaf area was heavily depressed in the lower leaves where the extreme wilting

Table 2. Comparison of leaf water content on dry weight basis (%) with that on leaf area basis (leaf water index *LWI* mg. H_2O/sq. cm.) in a turgid control and a wilted tobacco plant (see text). Leaf dry weight of unit leaf area (Leaf dry matter index *LMI* mg. d. w./sq. cm.) is also given.

Number of leaf node from the apex	Control Plant			Wilted Plant		
	leaf water content		*LMI*	leaf water content		*LMI*
	dry weight basis	*LWI*		dry weight basis	*LWI*	
2	558	23.0	0.41	—	—	—
3	567	22.1	0.40	503	24.4	0.49
4	612	21.5	0.35	634	22.4	0.35
5	641	21.1	0.33	651	20.6	0.32
6	638	22.2	0.35	669	20.1	0.30
7	707	22.6	0.32	655	17.9	0.26
8	—	—	—	689	17.9	0.26

could be observed, while the water content on dry weight basis did not indicate such clear depression. Another investigation whose main results were illustrated in Fig. 3 indicates that the leaf water amount in unit area was nearly constant in leaves of young tobacco plants after 4 weeks from sowing, despite of wide range of water content on dry weight basis, which fluctuated with the fluctuation of the leaf dry weight in unit leaf area.

From these facts, it is quite evident that the better fitness in expression of the leaf water is observed on leaf area basis rather than on dry weight basis (cf. also Koketsu), because with the latter the grading of leaf wilting can not be evaluated properly even in case of the visual wilting of plant. Moreover, the morphological characteristics of leaves relating to their water conditions are determined by two factors, i.e., the water amount and the dry amount in unit leaf area. Both factors influence also the dry matter production in the plant indirectly through determining the leaf thickness and assimilatory area, and they may in plant ecology be of importance enough to be designated in terms of leaf water index ($LWI=(W_f-W_d)/\overline{F}$, mg. H_2O/sq. cm.) and leaf dry matter index ($LMI=W_d/\overline{F}$ mg. d. w./sq. cm.), respectively.

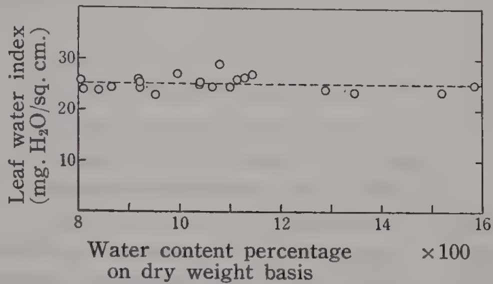


Fig. 3. Diagram showing the constancy of the leaf water index, in spite of wide variation of the leaf water content on dry weight basis.

Table 3 shows the values of *LWI* and *LMI* in different species computed from Monsi's data[6]). There are slight differences in *LWI* between evergreen broad-leaved trees and herbs, whereas in *LMI* the former show larger values than the latter in general. It is understandable, therefore, that the evergreen broad leaves have the following characters; the water content is considerably lower than that of herbs,

Table 3. Leaf dry matter index and leaf water index in various plants classified by life forms. Calculated from the data in Monsi 1944[6]).

Life form		Month measured	*LMI*	*LWI*
Deciduous broad-leaved trees	*Aphananthe aspera*	Sep.	6.0	9.9
	Sambucus Sieboldiana	Aug.	6.4	19.2
	Cornus controversa	Jun.	6.1	12.4
Evergreen broad-leaved trees	**Rhododendron hortense*	Jun.	7.8	12.2
	Evonymus japonica	Aug.	10.3	22.0
	Camellia Sasanqua	Apr.	15.6	17.7
	Ilex integra	Apr.	15.0	20.7
Herbs	*Impatiens Balsamina*	Aug.	2.5	20.2
	Mirabilis Jalapa	Jul.	3.3	24.4
	Vicia Faba	Jan.	2.2	18.2
	Helianthus annuus	Jul.	2.4	18.6
	Commelina communis	Jun.	2.1	17.1
Leaf succulent plant	*Sedum alboresum*	Aug.	8.9	102.1

* Summer leaf.

though the leaves develop in thickness. On the other hand, in a leaf succulent *Sedum alboresum* the large value of *LWI* and somewhat low *LMI* prove the well developed water storage organs in the leaves.

From the above facts, it is better to express leaf water content with that in unit area whenever the water economy of plants may be discussed in the interrelation between changes of the leaf water content and the transpiration from the leaf surface. Moreover, the expression of leaf water amount in the same unit as that of precipitation should make the quantitative analysis of water economy of plant community as a whole and the calculation of the quota for irrigation easier.

3. Formulation of the water economy:

The water deficiency of a plant under severe environmental conditions reveals itself chiefly in the leaf which transpires a large amount of water, while that of stem and root is scarce[3,7]. Therefore, except for extremely detrimental water conditions, there will generally be no extreme errors in discussion of water economy of an intact plant even if the water content of stems and roots is taken no account of in the equation discussed below.

In order to interprete the leaf water deficiency which was induced in the tobacco plant by the water level lowering, the following equation concerning the water economy in leaves was obtained with basic consideration of the equation after Montfort[1];

$$\text{Water balance} = A_t \cdot C_w - d \cdot T_1 \cdot \overline{F} \quad \ldots\ldots\ldots\ldots\ldots\ldots \ (1)$$

A_t is a coefficient of water absorption through unit fresh weight of root: C_w, fresh weight of roots active in water absorption (mg.): d, saturation deficit (cm. Hg.) of the surrounding air: T_1, transpiration rate (mg. H_2O/sq. cm./hr./cm. Hg) and $\overline{F}$ is total leaf area (sq. cm.). If the normal leaf water amount is represented by LWI_0, the actual leaf water amount of unit leaf area LWI is given by the following equation;

$$LWI = LWI_0 + (A_t \cdot C_w/\overline{F} - d \cdot T_1) \quad \ldots\ldots\ldots\ldots\ldots\ldots \ (2)$$

and when the value of $d \cdot T_1$ exceeds that of $A_t \cdot C_w/\overline{F}$, the water deficit of leaves will occur, that is,

$$\text{Relative water deficit (\%) in the leaf} = \frac{d \cdot T_1 - A_t \cdot C_w/\overline{F}}{LWI_0} \times 100 \quad \ldots. \ (3)$$

The main components in the equation, T_1 and A_t, will be discussed in the following.

a) The variation of transpiration rate with the active root/leaf area ratio.

Transpiration of entire plants with various water levels of culture solution was measured by a weighing method. The results thus obtained were converted into the unit of mg. H_2O/sq. cm./hr./cm. Hg after deduction of stem transpiration (14.1 mg. H_2O/g.f.w./hr./cm. Hg). No daily fluctuation in the transpiration rate was observed in the tobacco plants grown under the constant conditions of growth cabinet.

There are several works indicating the relation of transpiration of an entire plant to the total root/leaf surface ratio[8,9], showing some correlation between them. Here, however, in place of the ratio, the active root/leaf area ratio was used, because some parts of roots which have no ability to absorb water, e.g., taproot, are always included in the total weight of roots. As already reported[3], the transpiration rate was decreased in accordance with the progress of water deficit in leaves. Also the transpiration rate, if plotted against the active root/leaf area ratio, showed that any reduction of the rate did not occur under a normal leaf water condition with 4.5 to 7 of the ratio, but an almost linear depression in the transpiration rate was induced

by further reduction in the ratio (Fig. 4). The transpiration rate under normal leaf water conditions was in a range of 5 to 6 mg. H_2O (5.5 mg. in average)/sq. cm./hr. /cm. Hg. which was also indicated by another series of experiments.

b) Relation of the amount of submerged roots to the water absorption and transpiration.

In order to obtain the coefficient of water absorption through root surface, the total amount of absorbed water by a tobacco plant was determined volumetrically with a burette under a regulated condition of a growth cabinet (25°±0.5°, 1.2−0.9 cm. Hg) as hourly water loss from a small container. The materials used here were the same ones which were sampled for plant weight determinations (cf. Totsuka and Monsi[3]).

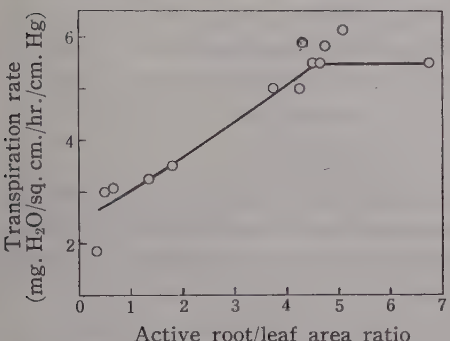


Fig. 4. Relation of the transpiration rate to the active root/leaf area ratio ($C_w/\overline{F}$).

There was a linear relationship between the amount of the submerged roots (C_w) and that of water absorbed (A), as shown in Fig. 5. The regression line (the coefficient of 0.96) calculated by the least square method is $A=0.885C_w+0.177$. Based on this, the value of the coefficient of water absorption A_t, could be estimated as 1.0 mg. H_2O/mg. f.w./hr. in average under the given conditions.

The amount of total transpiration from the shoot was investigated by a weighing method in parallel with the measurements of water absorption. The total transpiration showed as well a linear relation to the amount of submerged roots to coincide with the trend of water absorption (see also Fig. 5). This proves quite clearly the well known fact that when the water balance in a plant is kept normally, the amount of water absorbed through the root system is directly proportional to the amount of water transpired[10]).

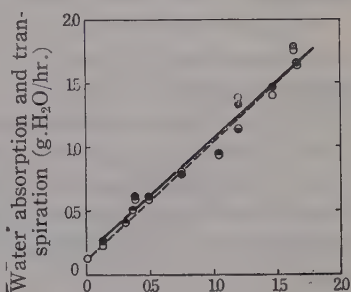


Fig. 5. Correlation between the submerged roots and the water absorption (solid circles and continuous line) and transpiration (open circles and broken line).

4. Comparison between the observed and the calculated water deficit of the leaf:

The variations of the relative leaf water deficit in response to the change of the active root/leaf area ratio $C_w/\overline{F}$ were calculated according to Equation 3. It was assumed here that the coefficient of water absorption of root A_t and the saturation deficit of surrounding air d were constant, i.e., 1.0 mg. H_2O /mg.f.w./hr. and 1 cm. Hg respectively, because the experiment was carried out under the constant conditions in the growth cabinet, and that the leaf water deficit varied with $C_w/\overline{F}$ and transpiration rate T_1. The changes of T_1 related with $C_w/\overline{F}$ were given in Fig. 4, and the normal leaf water index LWI_0 of 29 mg. H_2O was applied for the calculation[3]). The relative water deficit thus calculated is illustrated as a broken line in Fig. 6, against the $C_w/\overline{F}$ abscissae. The curve indicates similar tendency to the actually observed one (solid line in Fig. 6), though there were slight deviations of the calculated values from the observed ones. Such deviations may have principally been induced by the following two factors: (1) Some parts of capillary water ascending up the aerial parts of roots must be absorbed

by them, although this was neglected in calculation. (2) Here A_t was supposed to be constant, but soon after the water level lowering of culture solution, some promotion in water absorption should immediately occur to compensate the water deficiency of shoots. As to these problems further intensive studies are expected.

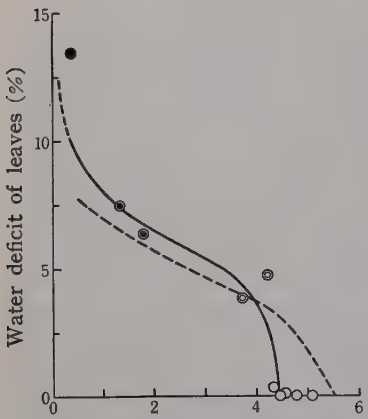


Fig. 6. Comparison between the observed water deficits of leaves (continuous line) and the calculated (broken line). Indications as in Fig. 1.

In conclusion, it can be said that Equation 3 will be applicable with a slight modification to the discussion of water economy of naturally grown plants, whose root development is restricted by some special causes, or is temporarily insufficient for offset of accelerated transpiration in the daytime.

Summary

1. The water economy of plants was studied in water-cultured tobacco plants reported in a previous paper[3]) by means of abrupt changing of culture solution level.

2. Equations 2 and 3 were formulated quantitatively to elucidate the water economy of plants, and they could clarify the main effective factors as follows:

a) The active root/leaf area ratio (the ratio of mg. fresh weight of root active in water absorption C_w to leaf area of plant in sq. cm. $\bar{F}$), which well indicated the grading of a water level lowering, had the direct effect on the water economy of an entire plant, and remained almost constant under normal conditions of the plant growth.

b) The transpiration rate kept a constant value of 5～6 mg./sq. cm./hr./cm. Hg in the range of 4.5～7 of the $C_w/\bar{F}$ ratio, but below 4.5 of $C_w/\bar{F}$, the transpiration rate decreased linearly with the depression of the active root/leaf area ratio.

c) The water amount absorbed through 1 mg. of submerged fresh roots was assessed to be 1 mg./hr. under the given conditions (25°, ca. 1 cm. Hg of saturation deficit).

d) The water amount in leaves was expressed on leaf area basis, and designated in terms of leaf water index (*LWI*, mg. H_2O/sq. cm.). The index represents the water condition of leaves more clearly than the water content expressed on dry weight basis does.

2) By substituting the above factors in Equation 3, the leaf water deficit of the tobacco leaves accompanied with lowering the culture solution level was calculated. The trend of the calculated curve was fairly similar to the observed. This may prove that the obtained equations will be applicable to the general discussion of water enocomy of plants with variously developed root systems.

References

1) Montfort, C., Zeitschr. f. Bot., **14**: 97 (1922). 2) Huber, B., Jahrb. f. wiss. Bot., **64**: 1 (1925). 3) Totsuka, T. and Monsi, M., Bot. Mag. Tokyo, **72**: 855 (1959). 4) Blackman, V. H., Ann. Bot., **33**: 353 (1919). 5) Koketsu, R., Journ. Dept. Agr. Kyushu Imp. Univ. **2**: 93 (1928). 6) Monsi, M., Jap. J. Bot., **13**: 367 (1944). 7) Livingston, B. E. and W. H. Brown, Bot. Gaz., **53**: 309 (1912). 8) Kramer, P. J., Handb. d. Pfl.-physiol., **3**: 188 (1956). 9) Parker, J., Plant Physiol., **24**: 739 (1949). 10) Kramer, P. J., Amer. J. Bot., **24**: 10 (1937).

摘　　要

水耕タバコにおける水分経済の解析

戸塚　績・門司正三

第一報で論じた水位低下によるタバコの乾量生長の減退にみられた水分経済の変化を解析するために，葉の水分欠差を算出する式を組み立てた（d は空気の水蒸気飽差 cm. Hg).

即ち，

$$\text{単位面積当りの水分欠差 (\%)} = \frac{d \cdot T_1 - A_t \cdot C_w/\overline{F}}{LWI_0} \times 100$$

葉の水分経済に影響する諸要因は次のようであつた．a) 水位低下の度合を表わす，根の生量に対する葉面積の割合 (active root/leaf area ratio=$C_w/\overline{F}$ ratio) が水分経済に著しい影響を与え，また一定の生育環境のもとではほとんど変らないことが認められた．b) 蒸散率 (T_1) は $C_w/\overline{F}$ ratio が 4.5〜7 では変化しないが，4.5 以下に低下するにつれてほぼ直線的に減少した．c) 水中部分の根の生量と吸収された水量との関係から得られた根の吸水能力 (A_t) は，根の生量 1 mg. 当り 1 mg. H_2O/hr. であつた．d) 水分経済を量的に論ずる際の葉の水分量表示としては単位面積当りの水分量が最適であつた．この含水量を特に“葉の水分指数”(leaf water index=LWI, mg. H_2O/sq. cm.) と命名した．

以上の結果を考慮し，上式を用いて水位低下の変化による葉の水分欠差を算出した結果，実測値とかなりよく一致した．従つてこの式は，一般に野外条件下で，土中の根の発達が著しい差異を示す場合の植物体の水分経済を論ずる手段として役立つものと考えられる。(東京大学理学部植物学教室)

Observation on the Apical Meristem of Rice Roots*

by Kei-ichi SHIMABUKU**

Received August 5, 1959

Since 1957 a comprehensive survey on the root system of the rice plants has been carried on by many investigators chiefly of the agricultural field, and the writer has undertaken a part of anatomical study. Although much work on the root apical meristem of the vascular plants has been done by a considerable number of investigators and many valuable contributions have been made, our knowledge on that of the rice plant is apparently insufficient. The present investigation was commenced with the purpose to get an accurate knowledge in the behaviours of apical initials and their derivatives in the promeristem, and, thus, to offer the reliable basis for a consideration of the tissue differentiation at higher levels and, on the other hand, of the determination of an appropriate length of the root tip for the chemical analysis and other studies.

Materials and Methods

Oryza sativa L. variety Norin No. 29 which was grown in seed beds or paddy-fields according to the ordinary method was used in this investigation. Seminal and adventitious roots were collected at various intervals, so as to obtain successive stages of development. Materials were killed and fixed in FAA, and dehydrated in normal butyl alcohol. Paraffin sections were usually cut at $10\,\mu$, and stained with a combination of safranin and Heidenhain's iron-alum haematoxylin or fast green.

Observation

In the present study, the root is conveniently divided into following zones: 1) the initial region composed of a group of initial cells; 2) promeristem including the initial region; 3) the meristematic zone which occupies between approximately 100 and 500 μ or more above the root tip***; 4) the zone of elongation; and 5) the zone of maturation. In the root tip, there are three sets of initials in many cases. One gives rise to the stele, the second to the cortex, and the third to the root cap (Fig. 1; Pl. I–A, B). In a few instances, however, the initials appear to be separated into four sets, that is, the stele, the cortex, the epidermis, and the root cap start from independent initials respectively (Figs. 2, 4; Pl. I–C).

The stelar initials always occur as a single layer of cells at the stele apex, the boundary between the stele and the cortex being sharply defined immediately behind the initials (Figs. 1, 2; Pl. I–A, B, C). In the median longitudinal section stelar initials are usually represented by two (Fig. 1), sometimes one or three cells. They are fairly variable in shape, mostly elliptical or polygonal, and they appear to divide

* Contributions from the Division of Plant Morphology, Botanical Institute, Faculty of Science, University of Tokyo, N. S. No. 83. This report was presented partly at the annual meeting of the Botanical Society of Japan, held in October 1958, at Kyushu University. Part of the cost of this investigation is paid by a grant from the Ministry of Agriculture and Forestry.

** Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

*** In this investigation, the root tip is represented by the group of initial cells and does not mean the tip of root cap.

in irregular directions. In the transverse section, increase in the number of stelar cells is not so remarkable in any directions at any levels, and growth of the cells, especially that of the parenchymatous cells, is also not conspicuous, but most stelar elements maintain the size of subdividing stage. Thus the stelar circumference expands but slightly and gradually. In the longitudinal sections, however, it is observable that frequent transverse cell divisions occur throughout the meristematic zone. Most of vascular elements become discernible at a level rather far from the stele apex, excepting the mother cell of the central vessel which is differentiated very early within 40 μ from the root tip. The pericycle is composed of one layer of cells which are almost equal in size and shape, and is interrupted by the protoxylem elements which are located in direct contact with the endodermis (Pl. I–E).

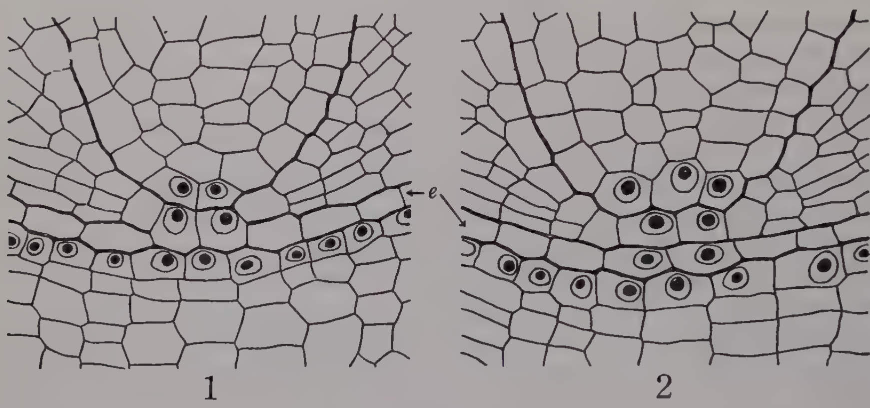
The number of protoxylem poles varies with root size. In the seminal root usually hexarch (Pl. I–E), while in the adventitious roots it fluctuates between 5–14, mostly 10 or nearly so. A protoxylem, as seen in Pl. I–E, occurs as a radially extending group of a few cells accompanied with an early-formed metaxylem vessel situated at the inner end of the row. The metaxylem is usually distinguishable by its relatively larger size (Pl. I–E) and differentiates before the protoxylem becomes visible. In the seminal and a number of the first-formed adventitious roots, the late metaxylem is represented by a single central vessel at the centre of the stele (Pl. I–D, E), while in the large later-formed adventitious roots, there are three or four central vessels which encircle a small pith (Pl. I–F). The central vessel differentiates foremost among the stelar elements, the early metaxylem follows, and the protoxylem is defined latest, while the maturation of elements takes place in reverse order. The first-formed protophloem is represented by a single sieve-tube which is diamond-shaped in cross sectional outline and is located immediately inside of the pericycle (Pl. I–E). Two small square cells contiguous to the two inner faces of it seem to be companion cells. The immature sieve-tube contains deeply stainable substanes, whereas in the mature one the contents become clear (Pl. I–E). The metaphloem cells are mostly round in shape, and larger than the protophloem cells.

Between the root cap initials and the stelar initials, usually are the cortex-epidermal initials which act as the common initials of the cortex and epidermis (Figs. 1, 3; Pl. I–A, B). In most cases, they are composed of two cells in the median longitudinal section (Fig. 1; Pl. I–A, B), occasionally one or three. So far as the present investigation concerned, there seems to be no correlation between the number of initial cells and the root size. The cortical subinitials and the epidermal subinitials are originated from the cortex-epidermal initials. The mode of cell division of the initials seems to be summarized as follows (Figs. 1, 3; Pl. I–B): ordinarily an initial produces first a derivative by an anticlinal division, and soon a periclinal division of the latter appears to follow; the inner cell thus formed becomes a cortical subinitial, and the outer one, an epidermal subinitial.

As has been mentioned above, occasionally the cortex and the epidermis appear to start from the independent initial zones, namely, the cortical initials and the epidermal initials (Figs. 2, 4; Pl. I–C). In such a case, the cortical initial zone is composed of two or three cells in the median longitudinal section. The mode of cell division of both initial zones resembles that of the cortical and the epidermal subinitials in the ordinary root tip (Figs. 3, 4).

The epidermal layer appears to be completed rapidly by frequent radial divisions of its subinitials and their derivatives near the root tip. In upper parts, however, the number of epidermal cells in transverse sections increases but gradually, for instace,

in a root it is 78, 87, and 99 at the levels 50, 100, and 200 μ respectively from the root tip. Thus, their number on the circumference is almost determined in earlier stages of the ontogeny. On the other hand, as clearly shown in the longitudinal



Figs. 1, 2. Median longitudinal sections through the initial region. Nuclei are shown in initial cells only, *e* epidermis, ×800. (Compare with Pl. A–C.) 1, ordinary feature of the initial region; 2, four-layered initial zone.

section, the epidermal cells repeat frequent transverse divisions throughout the meristematic zone. The periclinal divisions, however, could not be observed at any levels, thus the epidermis apparently consists of a single layer of cells (Pl. I–A, D). Within the level approximately 100 μ they suddenly increase their radial diameter, and then expand gradually in tangential direction at upper parts. In the zone of active cell division of the epidermis, the radial diameter is usually twice or three times as large as the tangential diameter (Pl. I–D). Elongation of the cells begins after they rapidly attain their full radial diameter in the meristematic zone.

The cortical subinitials rapidly produce the derivatives by their anticlinal divisions as in the epidermal subinitials, and the derivatives are soon turned into the meristematic endodermis by whose periclinal divisions all cortical elements are produced (Fig. 3; Pl. I–G). The following four zones are distinguishable in the cortex: 1) the outermost layer or the exodermis; 2) one or two layers of small cells which differentiate later into the sclerenchyma; 3) several layers of thin-walled cortical cells situated inside the future sclerenchyma; and 4) meristematic endodermis which is turned later into typical endodermis.

The periclinal divisions of the meristematic endodermal cells (Pl. I–G) occur mostly within 150 μ from the initials, though this distance varies to some extent according to the size of roots and the stage of development. The meristematic endodermal layer always produces its derivatives centrifugally, in other words, there is no centripital division. In most of cortical cells no further periclinal division occurs excepting occasional divisions in the future sclerenchymatous layer. Th enumber of cortical layers and that of the meristematic endodermal cells on the circumference vary considerably according to the root size.

The thin-walled cortical cells seldom or never divide themselves radially, since in transverse sections these cells usually show a regular radial arrangement (Pl. I–D), which, however, is sometimes disturbed by occasional radial cell divisions of the outer thin-walled cortical parenchyma and the meristematic endodermis (Pl. I–F). On the

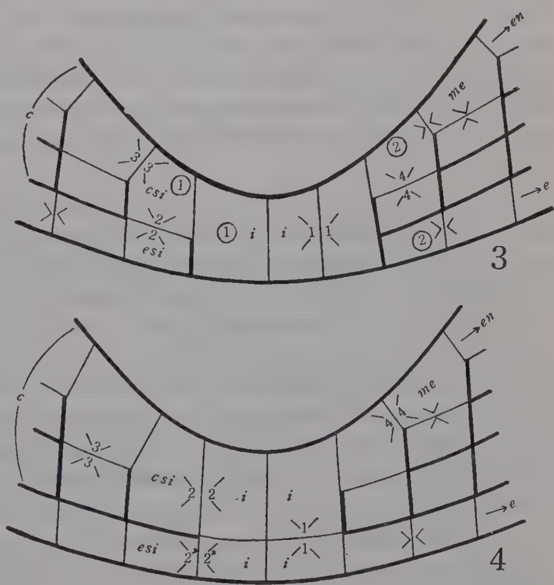
other hand, transverse cell divisions are frequent throughout the meristematic zone.

The exodermis is derived from the meristematic endodermis by its first periclinal division (Figs. 3, 4). In the meristematic zone and at the level where their growth begins, the exodermal cells apparently differ from the epidermal cells in shape and size (Pl. I–D), as well as in the manner of growth. In the zone of elongation of the root, the exodermal cells expand only slowly both in tangential and radial directions, thus they are almost equal in tangential and radial diameters in the zone of maturation.

Immediately beneath the exodermis there are one or in part two layers of cells which are the smallest of all the cortical cells, and contact tightly with each other (Pl. I–D, F). They turn finally into sclerenchymatous cells whose walls are thickened foremost of all the parts of the root. They arise from the meristematic endodermis after the exodermis, and enlarge slightly and gradually in all transversal directions, while they divide in radial, occasionally also in tangential direction. They maintain their squarish or polygonal outline and almost uniform throughout their developmental stages.

Most parts of cortical layers are occupied by the thin-walled parenchymatous cells arranged in regular radial rows in the transverse section (Pl. I–D). These cells are derived last from the meristematic endodermis by its periclinal divisions. They are at first very flattened rectangles in shape and later become elliptical, when diamond-shaped intercellular spaces appear at their corners (Pl. I–D, F). These spaces become visible in the outer layer of the thin-walled parenchyma at the level approximately 50 μ or less from the root tip where the root cap still persists, and formation of the spaces proceeds centripetally. Cells of both the inner and the outer layers of the thin-walled cortical parenchyma maintain more deeply stainable contents for a long time in contrast with those of the middle layer (Pl. I–D). The cell walls of these cells are slightly thickened when the root becomes mature. These cells maintain the shape after the other thin-walled cortical cells are ruptured eventually by the formation of large air spaces.

In the median longitudinal sections observed, the root cap initials are ten or nearly so in number. Cell divisions of these initials take place periclinally, but rarely anticlinally, and further periclinal divisions of their derivatives form a central core with regular parallel rows of cells. On the other hand, at the periphery of the cap the derivatives of the initials are divided periclinally



Figs. 3, 4. Diagrams of median longitudinal sections of the cortex-epidermal initials showing cell divisions. 3, ordinary feature of the cortex-epidermal initials; 4, separated initials by periclinal divisions: *i* initial cell, *csi* cortical subinitial, *esi* epidermal subinitial, *me* meristematic endodermal cell, *c* cortex, *en* endodermis, *e* epidermis; numbers indicate the order of cell divisions. Further explanations in the text.

as well as anticlinally, thus the arrangement of the cells in the outer periphery of the cap differs, from that of the central core.

Discussion

As has already been shown by many investigators[1,2,3]), initials of rice root are usually composed of three distinct layers, namely, the stelar, the cortex-epidermal, and the root cap initial layers respectively. The similar arrangement of initials was described by Heimsch[4]) in *Hordeum*, and by Clowes[5]) in *Triticum* and *Zea*, and furthermore according to Schüepp[6]), there are many other monocotyledons including many grasses with similar organization. Thus the usual organization of the apical meristem in the rice root should be regarded as the type most common among the Graminae.

Clowes[7]) has noted that in the majority of median longitudinal sections of *Zea* and *Triticum* roots, two cells occurred at the pole of the periblem-dermatogen complex; the pole of apical meristem which has been called ‘quiescent centre’, is composed of cells which are either nondividing or slowly dividing. His statement has been supported by Jensen and Kavaljian[8]), who noticed in the root tip of *Allium cepa* that no divisions were observed in the region of apical initial group.

In the rice roots, however, it was ascertained that the cortex-epidermal initials seem to maintain the ability of cell division. Usually the cortex-epidermal initials are divided at first anticlinally and then the derivatives divide periclinally to form the cortical and the epidermal subinitials. One of the reasons that the alteration in number of the polar cells may be due to the difference of the stage of cell division. After the periclinal division of the derivatives is finished, the cortex-epidermal initials appear to consist of two cells and at that stage they attain maximum size (Figs. 3, 4). Thus the cortex-epidermal initials may be fundamentally composed of a small number of cells.

In a few instance, however, the initial region appears to be separated into four layers: the stelar, the cortical, the epidermal, and the root cap initial layers. Clowes[5]) has noted that the pole of apical meristem may play a cytogenerative role when the architectural pattern is upset after wounding. According to Pellegrini[9]), the action of promeristem is conditioned by a certain minimal number of initials in his surgical experiment. Kaufman's experiment[3]) should be noticed in this connection. He has observed in rice that adventitious roots in 2, 4-D-treated shoots differ from those of control plants in the behaviour of the protoderm-cortex initials. Instead of dividing only anticlinally as in untreated root apices, these cells divide both anticlinally and periclinally and give rise to several layers at the summit of the protoderm-cortex zone, whereas in the other initials any morphological effect is not found. The fact in his observation may suggests that, in rice roots, at least the cortex-epidermal initials retain an ability of the periclinal division, and the cytogenerative role of these initials is possibly occurred under certain conditions. From the fact that in the present study the four-layered initial zone is found only among relatively large later-formed adventitious roots, it may be conceivable that the latent ability of the periclinal division is brought to light by the increase of root size. Heimsch[4]) has observed in barley roots that between the caryptorogen and the stelar initials there are the common initials of the cortex and epidermis, and they are one or two cells deep in those poles. Though he gave no explanation on the cause of the alteration of the number of initial cells, his observation suggests that the same phenomenon as in the case of rice

plants is also present in barley, and further that the constructional types of roots in vascular plants may be changeable under certain conditions.

According to Heimsch[4]) the adventitious roots of barley seem to be characterized by several central vessels. Their number in the rice, however, varies with size of roots. In small early-formed adventitious roots, the late metaxylem is represented by a single central vessel as in the case of the seminal root.

Juliano and Aladama[10]) have observed that the pericycle in rice plants is mostly continuous, though rarely it is interrupted by the protoxylem. In the present study, however, the latter condition is always observed. According to Guttenberg[11]), the latter type of pericycle seems to be quite usual in the Graminae.

Williams[12]) has indicated that in the tip of the primary root in many vascular plants the cell layer which becomes the endodermis finally, acts like a cambium giving rise to all the tissues between the endodermis and the exodermis (he called it 'hypodermis'). The cambial nature of endodermal cells was confirmed also in this study. All the cortical cells, namely, exodermis, sclerenchyma, thin-walled cortical cells, and endodermis, are of the same origin, though they are clearly distinguishable in shape, size, manner of division and growth, as well as in final wall thickeness.

Judging from the staining reaction, shape, and number of cells of each layer of the cortex mentioned above, it seems quite apparent that the cells of outer layers of the cortex and the endodermal cells maintain for a long time an ability of the anticlinal division. Disturbance of regular radial rows of cells in the thin-walled cortical parenchyma seems to depend upon anticlinal divisions of the cells of its outer layer and the endodermal cells. It is also confirmed that most of the cortical cells maintain no ability of periclinal division excepting the future sclerenchymatous cells in which such divisions occur occasionally, and that the cells of middle portion of the thin-walled cell layer of the cortex have no ability of division in any directions. The level at which the diamond-shaped intercellular spaces appear agrees with Boeke's observation[13]).

According to Clowes[6]), there are two types of organization in root apices of grasses, so-called *Triticum* type and *Zea* type. He noted that the difference between the two types of organization depends chiefly upon the patterns of division within the cap, and can possibly be related to the difference in size of the apices. The general pattern of the rice root resembles *Zea* type.

I wish to express my most grateful thanks to Dr. S. Watari for his continous directions and constant encouragement throughout the investigation, and also to the members of my laboratory for their valuable advice. My thanks are also due to Dr. R. Aimi of the National Institute of Agricultural Science, Tokyo, for valuable materials.

Summary

The fundamental organization of the root tip of *Oryza sativa* L. was observed and discussed with special reference to the bevaviour of the initial cells and their derivatives in the promeristem.

Usually the promeristem has three sets of initial layers, i.e., the stelar, the cortex-epidermal, and the root cap initials, thus the constructional type of promeristem being conformable to the most common pattern of grass roots.

In a few instances, however, the initials appear to be separated into four sets, that is, the stele, the cortex, the epidermis, and the root cap being derived from an

independent group of initials respectively. Existence of two types of organization in one and the same species under a natural condition is very interesting, and probably has not been noticed by any workers.

It seems rather natural to consider that the initial cells maintain an ability of division. The cortex-epidermal initials which divide anticlinally, may occasionally divide also periclinally to form a four-layered initial zone. This fact seems to furnish an evidence of the latent ability of periclinal division of cortex-epidermal initials.

The cambial nature of endodermal cells is confirmed, that is, all of the cortical cells are the same in origin, though among the cells they differ in shape, size, dividing ability, as well as in the manner of division and elongation.

References

1) Haan, J. Van B. De, De Rijstplant 1. (1911). 2) Yung, C. T., Bot. Gaz. **99**: 786 (1938). 3) Kaufman, P. B., Amer. J. Bot. **42**: 649 (1955). 4) Heimsch, C., Amer. J. Bot. **38**: 365 (1951). 5) Clowes, F. A. L., New Phytol. **53**: 108 (1954). 6) Schüepp, O., Meristeme. In: K. Linsbauer, Handbuch der Pflanzenanatomie. Bd. 4. Lief. 16. (1926). 7) Clowes, F. A. L., New Phytol. **55**: 29 (1956). 8) Jensen, W. A. and L. G. Kavaljian, Amer. J. Bot. **45**: 365 (1958). 9) Pellegrini, O., N. S. Bull. dell' Ist. ed Orto Bot. dell' Univ. Napoli. **10**: 187 (1957). 10) Juliano, J. B. and M. J. Aladama, Philippine Agriculturist **26**: 1 (1937). 11) Guttenberg, H. von, Die physiologischen Scheiden. In: K. Linsbauer. Handbuch der Pflanzenanatomie. Bd. 5. Lief. 42. (1943). 12) Williams, B. C., Amer. J. Bot. **34**: 455 (1947). 13) Boeke, J. E., Ann. Jard. Bot. Buitenzorg **50**: 197 (1940).

摘　　要

イネの根の頂端分裂組織の観察

島　袋　敬　一

イネの根端の組織の基本的構造，特に前分裂組織における始原細胞群の行動，ならびにそれに由来する細胞の分裂方向について観察し論議した．

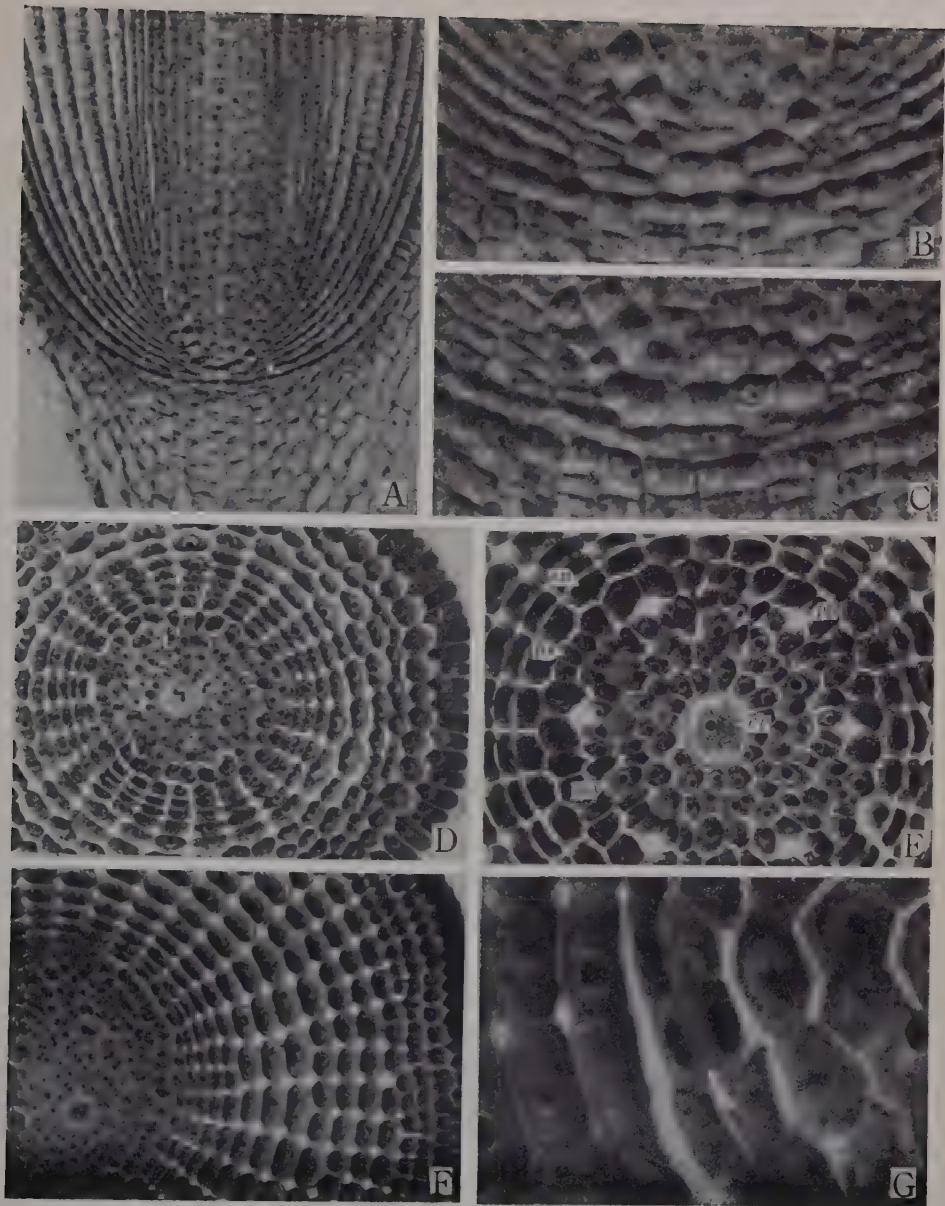
多くの場合，前分裂組織は縦断面で中心柱，皮層・表皮，および根冠の3始原細胞群として区別でき，その構造はイネ科に普通にみられる型に属する．

しかし，少数例ではあるが，始原細胞群が4層――中心柱，皮層，表皮，根冠――にわかたれ，おのおのの独立の始原細胞群に由来していると考えられる場合がある．1個の種において，このように根の前分裂組織の構造が2型を示すことは興味ある問題であり，このことについては従来観察例が極めて少く，殆んど論議されてない．

近来，根の前分裂組織の細胞分裂の頻度，ならびに始原細胞群の分裂能力等について種種論じられている．この研究での観察例は，始原細胞群が分裂能力を持つことを示し，特に皮層・表皮始原細胞群は縦断面で anticlinal division を行うのが普通のようであるが，時に periclinal division の能力を発現することによつて皮層・表皮の共通の始原細胞群が分離し，おのおの独立の起原より生じ4層となつたと考えるのが至当であろう．

内皮の分裂能力についても観察した．皮層のすべての細胞は分裂帯の内皮細胞の periclinal division によつて形成される．しかし，各部位，すなわち内皮，薄膜柔細胞層の内，中，外層，厚膜細胞および外皮の各細胞はその形，大きさ，内皮細胞より分離した後の分裂能力ならびに分裂，伸長の方法が異なる．

(東京大学理学部植物学教室)



A–C. Median longitudinal sections of the root tip (cf. text figs. 1, 2). A, ordinary feature of the root tip, ×240; B, a part of A, under a higher magnification showing a part of promeristem with three-layered initial zone, ×800; C, four-layered initial zone, ×800.

D–G. Transverse sections. D, seminal root, showing the level 160 μ from the tip, note the shape of the epidermal cells, the arrangement of the cortical cells, and staining reaction of the cortex, ×230; E, level 280 μ from the tip, under a higher magnification, showing an upper part of the same root as D, ×430, *en* endodermis, *px* protoxylem, *mx* metaxylem, *pp* ptotophloem, sieve-tube elements become clear and are mature in E, immature in D, *cv* central vessel; F, adventitious root, level 400 μ from the tip, showing the four central vessels, and disturbance of radial rows of the cortical cells, ×240; G, level 30 μ from the tip, showing the periclinal division of the meristematic endodermal cell under a higher magnification, an arrow indicates the division, ×1,600.

K. SHIMABUKU: Observation on the Apical Meristem of Rice Roots.

植物細胞に対する凍結乾燥法の利用

三木寿子*・山岸秀夫*

Hisako MIKI* and Hideo YAMAGISHI*: Application of Freeze-drying Method to Plant Cells.

1959 年 8 月 5 日受付

Altmann[1]) により始められ，Gersh[2]) によつて改良された細胞に対する凍結乾燥法は，細胞を低温で凍結し，つぎに高真空中で水分を昇華させて細胞を乾燥させ，適当な包埋剤で包埋するものである．従来の固定法では，固定剤と脱水に用いるアルコールの影響のため，組織化学的研究にはいろいろの障害があつた．本方法はこの点の障害を克服するものといわれている．Mancini[3]) はグリコーゲンについて，Emmel[4])はフォスファターゼについてこのことを示している．しかし多くの研究者は動物材料を使つており，植物細胞では，Goodspeed and Uber[5]) が *Lilium longiflorum* の葯および *Allium cepa* の根端を用いて細胞学的検討を行なつて以来ほとんど行なわれていない．筆者らは動物材料の場合と同様，植物材料に対しても本方法が組織化学的にも，形態学的にもすぐれているかどうかを研究した．

材料および方法

形態学的研究のためには，*Spirogyra ellipsospora*, *Hydrodiction* sp., *Cladophra* sp., *Oedogonium* sp. および *Vaucheria* sp. を用いた．なかでも *Spirogyra* を主として用いた．これは固定によつて葉緑体の形が鋭敏に変化するので，葉緑体の細かい変化を観察することによつて，固定法の良し悪しの判別ができるからである．

組織化学的研究のためには，*Lilium longiflorum* の子房を用いた．これは，これまで筆者らの行なつて来た，この子房を 10% ホルマリンで固定してパラフインに包埋した材料および新鮮材料における組織化学的研究の結果と比較するためである．

凍結乾燥に用いた装置は第 1 図に示されている．実験方法としては，液体空気で冷却して少々粘稠になつたイソ・ペンタン（約 −150°）の中になるべく小さく切り出した材料片をいれて約 10〜30 秒間凍結させる．組織の場合は厚さ約 0.5 mm. に切る．凍結された材料は，約 −50° にあらかじめ冷却されている資料室 A の中にすみやかに入れ，排気する．乾燥時間は，Müller[6]) および Patten and Hopkins[7]) の報告した乾燥曲線を参考にして約15時間行なつた．この期間中の真空度は 10^{-3} mm. Hg 以下である．終末乾燥は，材料の乾燥状態から経験的に割り出して，約 2 時間で充分である．最後にパラフイン室 C にいれてあるパラフインをとかして資料室 A にそそぎ材料を包埋する．グリセリンまたは流動パラフィンで包埋するときは，これらを液体空気を用いて，あらかじめ

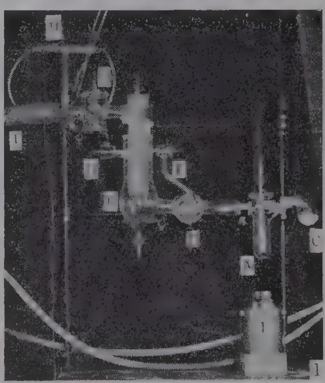


Fig. 1. Freeze-drying apparatus. A; Material chamber. B; Cold trap. C; Paraffin chamber. D, E, F, G; Cock. P_2O_5 trap is between G and F. H; Geisler tube. I; Vaccum bottle. J; Rubber tube connected with glass system and diffusion pump.

* Cytological Laboratory, Botanical Institute, Faculty of Science, Kyoto University, Kyoto, Japan. 京都大学理学部植物学教室

凍結させて資料室の底にいれ，その上に凍結させた材料をのせて乾燥させる．その後資料室の温度を室温にもどせば，材料はこれらの物質に包埋される．

結　果

1, 凍結乾燥法により処理された材料の形態学的研究．

a. 新鮮な細胞と凍結乾燥法により処理された細胞との比較．　凍結乾燥法のみの影響でどのように藻体の形態が変化するかをみるために，凍結乾燥後真空でグリセリンに包埋したものと新鮮材料との比較を行なつた．

種々の材料の中で *Spirogyra* の固定が困難であるので，これについてとくに報告する．*Spirogyra ellipsospora* の新鮮材料は1細胞内に3～8個の葉緑体を持つており，この細長い葉緑体の両側には鋸歯状突起がある（第 2a 図）．細胞核は原形質糸によつて細胞の中央に保たれ，核には大型球形の仁が1個認められる（第 3a 図）．凍結乾燥法を行なつたものでも葉緑体の鋸歯状突

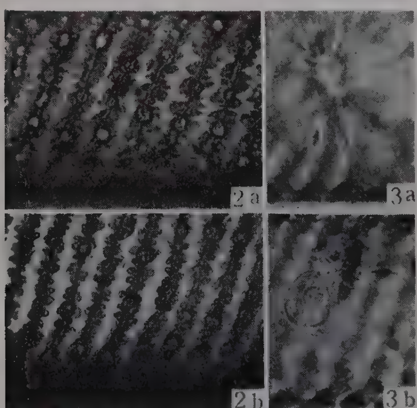


Fig. 2. (a) Fresh chromatophores (*Spirogyra ellipsospora*). (b) Freeze-dried chromatophores mounted in glycerin *in vacuo*. The serrated margins are well preserved.

Fig. 3. (a) Fresh nucleus. (b) Freeze-dried nucleus mounted in glycerin *in vacuo*. The strand of cytoplasm are disappeared.

起は変形していないし，葉緑体の切断も全く見られない（第2b図）．このような細胞では細胞核の周囲の原形質糸の保存がよくない．また仁は新鮮材料におけるものよりも明瞭に認められる（第3b図）．

さらに凍結乾燥法により処理された藻体が，その後の染色，脱水およびバルサム封入などの影響でどのような形態変化をするかを研究した．凍結乾燥後の処理法にはつぎの二つの方法を用いた．(1) 藻体を凍結乾燥し，さらに真空中でグリセリンに包埋した後，装置から取り出し，グリセリンを水で除いた．つぎに藻体をハイデンハイン・ヘマトキシリンで染色をし，アルコールで脱水した後，カナダバルサムに封じた．これを便宜上，凍結乾燥A法とよぶ．(2) 藻体を凍結乾燥後常圧にもどし，ホルマリン蒸気で固定を行ない，アルコールを経て水に移し，以下 A 法と同じ染色・脱水・封入を行なう．これを凍結乾燥B法とよぶ．A法で処理された *Spirogyra ellipsospora* の細胞では，葉緑体の鋸歯状突起はほとんど変形していないが葉緑体の切断が多数認められる．また細胞壁の破壊は少ない（第4a図）．B法で処理された細胞について葉緑体を見ると，鋸歯状突起の形態は保たれているが，切断箇所はA法によるものよりも多い（第 4b 図）．また細胞壁はほとんど破壊されていない（第 4c 図）．この場合，上のようにB法で処理されバルサムに封じられた細胞を，カバーグラスの上から押しつけると，細胞壁・葉緑体はあたかもガラス片が粉砕されたように，容易に細かく破壊される（第5図）．これは，凍結乾燥により可塑性が減じて細胞が結晶状になつたからであろう．またA法またはB法で処理された核は他の固定液で固定・染色された核よりも，核内要素の分染が著しくすくない（第6図）．

b. 凍結乾燥法により処理された細胞と種々の固定液で処理された細胞との比較．

形態の比較　上記 a で述べたように，凍結乾燥法は，細胞の形態にあまり大きな変化を与えずに細胞をバルサムに封じることの出来るすぐれた処理法である．しかし他の種々の固定法の中に，凍結乾燥法よりもすぐれて新鮮細胞の形態を，バルサムの中に封じるまでよく保存するものがあるかどうかをしらべた．したがつてここでは形態に与えられている変化は，固定のみの影響でなく固定後の処理の影響も加算されているものとみなされる．

ここでは，凍結乾燥法としてはA法とB法とを用いた．なお，ここで用いた固定液は，special

chromo-acetic-osmic solution*・strong chromoacetic solution**・1% オスミウム・5% ホルマリン・formo-acetic alcohol*** およびナワシン固定液である．

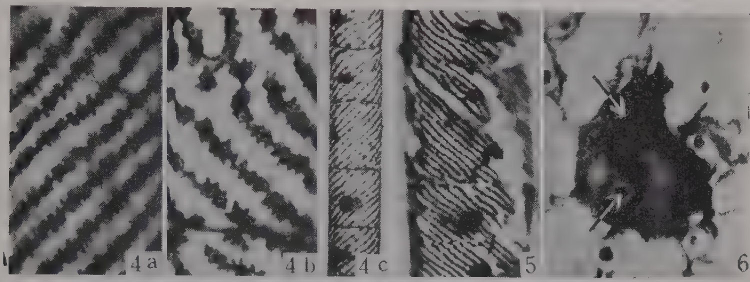


Fig. 4. (a) Freeze-dried cell stained with Heidenhain's haematoxylin after mounted in glycerin *in vacuo*. The method used here is designated 'F.D.–A' in this paper. The chromatophores are not segmented and their serrated margins are well preserved. (b) and (c) Freeze-dried cells were fixed by formalin vapor and stained with Heidenhain's haematoxylin. The method used here is designated 'F.D.–B' in this paper. The chromatophores are segmented, the serrated margins of chromatophores are well preserved and the cell wall is not damaged.

Fig. 5. The cells are crushed by a slight pressure given on a cover glass soon after the cells are freeze-dried, stained and mounted in balsam. Cell walls and chromatophores are fragmented.

Fig. 6. A freeze-dried nucleus treated by the F.D.–B method. Nucleoli are not clear. Arrows indicate pyrenoids.

固定の良否を比較する目的で，便宜上細胞壁と葉緑体の形態を基準にした．とくに葉緑体の鋸歯状突起は固定の影響によつて鋭敏に変化するから規準として適当である．固定による変形の状態を便宜上 A・B・C・D・E および F の6種類に区別した．この場合，Aは細胞壁が破壊されず，葉緑体の切断もなく，鋸歯状突起がよく保存されているものである（第 4a 図）．B は葉緑体に切断箇所が見られる以外はAとおなじ種類のものである（第 4b 図）．C は細胞壁が破壊されず葉緑体の切断はないが，鋸歯状突起が著しく変形しているものである（第 8b 図）．D は葉緑体に切断がみられる以外はCと同じ種類であり，E は細胞壁が破壊されているものである．F は細胞全体が不規則に収縮しているものである．そこでそれぞれの固定法の場合に，バルサムの中ではどのような状態に保たれている細胞が多いかを，ヒストグラムにして第7図に示した．

第7図の結果をみると，凍結乾燥後，真空中でグリセリンに包埋した後，染色を行なつたもの（A法）では，B法によるものと比べてAのみられる頻度が多く，Bのみられる頻度が少ない．しかしいずれも葉緑体の鋸歯状突起はよく保存されている．他の固定法では，special chromo-acetic-osmic solution 以外は葉緑体の鋸歯状突起の保存は悪く，AおよびBに属するものはほとんどみられない．この点では凍結乾燥法とともに，special chromo-acetic-osmic solution 法もかなりすぐれているといえる（第 8a 図）．組織化学的研究によく用いられるホルマリン固定法では，鋸歯状突起は著しく変形している（第 8b 図）．また special chromo-acetic-osmic solution およびホルマリンで固定された核では，A法およびB法で処理されたものと異なつて，仁と仁以外の核の要素とはよく分染される．また原形質糸の保存はよくない（第 9a, b 図）

* Chromic acid 1 g.: Glacial acetic acid 3 ml.: 1% osmic acid 1 ml.: Water 100 ml. (modified by Chamberlain[3]).

** Chromic acid 1g.: Glacial acetic acid 3ml.: Water 100 ml.

*** 50% alcohol 90 ml.: 40% formalin 5 ml.: Glacial acetic acid 5 ml.

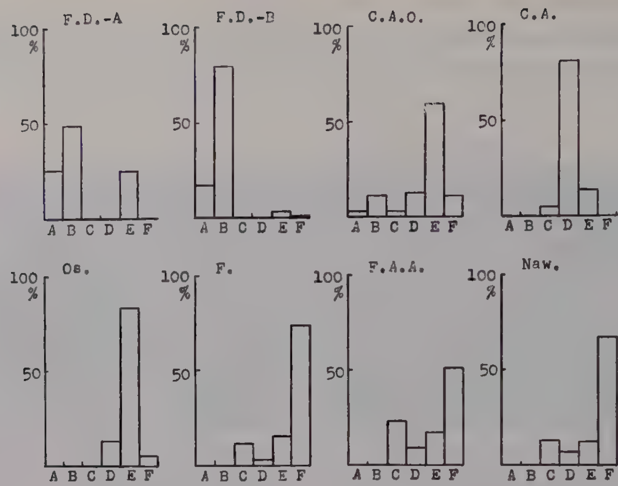


Fig. 7. Histograms show the relation between the grades of deformation and the frequency of their occurrence. The grades of deformation are indicated by A, B, C, D, E and F as follow: A; The cell wall is not damaged, the serrated margins of chromatophores are well preserved and the chromatophores are not segmented. B; Similar to (A) except the segmentation of chromatophores. C; The cell wall is not damaged and deformed. Serrated margins of the chromatophores are hardly visible. Chromatophores are not segmented. D; Similar to (C) except the segmentation of chromatophores. E; Cell walls are deformed. F; Cells are irregularly contracted. F. D.–A and F. D.–B; See in explanation of Fig. 4. C.A.O.; Special chromo-acetic-osmic fixation. C.A.; Strong chromo-acetic fixation. Os.; Osmic acid fixation. F.; Formalin fixation. F.A.A.; Formalin acetic alcohol fixation. Naw.; Nawaschin fixation.

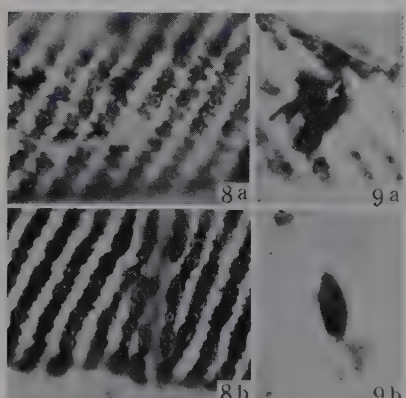


Fig. 8. (a) Chromatophores fixed by special chromo-acetic-osmic solution, stained with Heidenhain's haematoxylin, and mounted in balsam. The chromatophores are segmented while the serrated margins are well preserved. (b) The chromatophores fixed by 5% formalin, stained with Heidenhain's haematoxylin and mounted in balsam. The chromatophores are not segmented while the serrated margins are little preserved.

Fig. 9. (a) Nucleolus treated by the same procedure in Fig. 8(a); the nucleolus is distinct. The arrow indicates the nucleolus. (b) A nucleus treated by the same procedure as Fig. 8(b); the nucleolus is distinct.

葉緑体の切断数　上にのべたように，凍結乾燥された細胞や種々の固定液で固定された細胞を，染色・脱水した後バルサムに封じると，細胞壁は破壊されなくても葉緑体には，かなりの切断がみられることがある．そこで1細胞あたりの葉緑体の切断数をしらべてその多少を比較した．その結果は第1表に示されている．

第1表の結果によると，A法，B法および

Table 1. Number of segmentation of chromatophore per cell.

Fixation	F.D.–A	F.D.–B	C.A.O.	C.A.	Os.	F.	F.A.A.	Naw.
No. of segmentation	1.13	2.74	2.81	2.84	/	0.68	0.30	0.75

special chromo-acetic-osmic solution 法に葉緑体の切断数が多い．とくにA法と比べるとB法では切断数が多い．これとちがつて鋸歯状突起の保存されにくいホルマリン固定や formo-acetic alcohol 固定によるものでは，切断数が少ない．

細胞容積の変化 一般に藻類の細胞では，固定後の脱水・透明剤処理・バルサム封入などの段階で，いちぢるしく細胞容積が収縮するのが普通である．*Spirogyra* の場合に，この収縮がどの段階ではげしいかを，種々の固定液によるものと，B法によるものとについて比較した．

藻体をB法で処理したものと，数種の固定液で固定しさらに染色・脱水・透明剤処理・バルサム封入の諸段階を経た後の細胞容積を新鮮材料の細胞容積を 1.00 として比較した．その結果は第2表に示されている．

Table 2. Change of cell volume after fixation.

Preparation	F.D.–B	C.A.O.	C.A.	Os.	F.
After fixation	1.15±0.03	0.90±0.02	1.00±0.03	0.97±0.03	0.97±0.03
In xylol after dehydration by ethanol			0.91±0.03		
Immediately after mounting in balsam	1.04±0.03	0.71±0.02		0.85±0.03	0.79±0.03

Cell volume of fresh material is assumed as 1.00.

第2表の結果によると，凍結乾燥B法で処理したものは，新鮮材料と比べて著しい細胞容積の変化は認められない．しかしこの場合，凍結乾燥直後には，むしろ容積が増加していることは注目に値する．この増加した細胞容積は，後の脱水・バルサム封入などの操作中に収縮して，もとの新鮮材料の細胞容積に近づく．これと異つて，種々の固定液によつて細胞を固定し，染色後バルサムに封入すると，著しい容積の減少がみられる．とくに special chromo-acetic-osmic solution ではこの傾向がつよい．

2. 凍結乾燥法により処理された材料の組織化学的研究

従来の植物組織の組織化学的研究法には，新鮮材料をそのまま切片にして用いる方法と，ホルマリン固定をしたもののパラフインまたはセロイジン切片を用いる方法などがある．これらの方法では，操作中に組織内の特定物質が移動したり，溶け出したりするおそれがある．したがつて本研究では，とくに反応の部位と反応の有無について，凍結乾燥法によるものと，従来の方法との比較を行なつた．

Lilium longiflorum の開花当日の子房を，凍結乾燥法によりパラフィンに包埋したものを 25μ の厚さに切り，スライドにはりつけ，10分間ホルマリン蒸気にあてて固定する．これを脱パラフィンし，第3表に示すような組織化学的試験を行なつた．この場合ホルマリン蒸気で固定しないで，組織化学的試験を行つたものと比較したが，反応の相違は認められなかつた．本研究では，凍結乾燥をしたものと，新鮮組織を 150μ の厚さの切片にして，直ちに反応を行なつたものと，10%ホルマリンで固定してパラフィンに包埋し，25μ の厚さに切つて脱パラフィンし，反応を行なつたものとの比較をした．この試験ではそれぞれの方法においての反応の有無と，反応の部位などを比較した．この結果は第3表に示されている．

この結果によると，検出法のちがいによつて，反応の有無および部位のちがいの認められるものがあつた．たとえば，ヨード反応および Millon 反応などでは，反応の有無および部位が，上記の新鮮組織，凍結乾燥法および 10% ホルマリン固定法によるものとの間では，差が認められなかつた．

これと異なつて，Sudan III 染色では，新鮮組織について調べると，柔組織では1細胞あたりに 1～2 滴の油滴が scarlet red* に染まるのが認められ，表皮や子房内壁およびはいしゆでは，細胞全体に拡散して grenadine red に染まり，滴はみえない．またはいしゆではは いのうに近いほど濃く染まる．とくにはいのうは最も濃くそまる．

* 色の記載はすべて Ridgway, R. etc,: Color standards and nomenclature. Washington, D. C. (1952) を参照した．

凍結乾燥をした材料および 10% ホルマリンで固定をした材料では，柔組織内の油滴は全然認められない．表皮や子房内壁およびはいしゅでは，凍結乾燥法と新鮮組織のものとの間では，いちぢるしい差は認められない．これと異なつて，10%ホルマリン固定のものでは，はいのうだけが grenadine pink に染まり，他の部分は染まらない．これは固定後の処理により，脂溶性のものが溶け出したものと考えられる．

また，Fehling 試薬による還元糖の反応についていうと，新鮮組織および凍結乾燥法によるものでは，はいのうが rose pink に染まるのがみられる．ところが 10% ホルマリン固定をしたものでは，陽性反応はほとんど認められなかつた．これは還元糖もまた固定後の操作によつて溶け出したためであろう．

上の場合に似た結果を示すものは benzidine 反応の場合である．この反応では，新鮮組織および凍結乾燥法によるものでは，はいのうに顕著な陽性反応が見られるが，10% ホルマリン固定のものでは陰性であつた．また M–nadioxidase 検出法では，凍結乾燥法および 10% ホルマリン固定法によるもののどちらも，子房の表皮およびはいしゅに反応が認められるが，凍結乾燥を行なつたものの方がホルマリン固定をしたものよりも強い反応を示した．

上の場合とちがつて，オスミウム酸の還元反応，–SH 検出法，G–nadioxidase 検出法およびこはく酸脱水素検出法では，新鮮組織においてのみ反応がみられ，凍結乾燥法で処理したものおよび 10% ホルマリンで固定をしたものでは，反応は陰性であつた．なお Feulgen 反応では，凍結乾燥法で処理したものおよび 10% ホルマリンで固定をしたものでは，核または染色体が鮮やかな陽性反応を示した．

なお，反応部位が他の固定法による場合と全然異なるというような反応は，今のところでは観察されていない．

Table 3. Histochemical tests in the ovary of *Lilium longiflorum*.

Histochemical reagent	Method of treatment			Locality of reaction
	No treatment (fresh)	Freeze-dried	10% formalin fixation	
Jod-jod kali	+	+	+	Parenchyma: Ovule
Sudan III*	⧺	⧺	+	Epidermis: Inner ovary wall: Ovule
Osmic acid	+	−	−	Epidermis: Inner ovary wall
Millon's reagent	+	+	+	Epidermis: Ovule
Na-nitroprusside	+	−	−	Epidermis: Ovule: Vascular bundles
Fehling's reagent	⧺	⧺	+	Embryo sac
Feulgen's reagent		+	+	Nucleus: Chromosome
M-nadi reagent		⧺ bluish	+ violescent	Epidermis: Ovule
G-nadi reagent	+	−		Embryo sac
Benzidine	+	+	−	Embryo sac
Na-succinic acid and methylen blue	+	−	−	Parenchyma: Embryo sac

+: Positive reaction. −: Negative reaction.
*: Acetic-carbol-Sudan III reaction by Jackson procedure (Jackson, C.[9])).
**: Variation of locality.
Non treated tissue: parenchyma, epidermis, inner ovary wall and ovule.
Freeze-dried tissue: epidermis, inner ovary wall and ovule.
10% formalin fixed tissue: embryo sac.

論議および結論

凍結乾燥直後，真空中でグリセリンに包埋された *Spirogyra* の葉緑体と，A 法およびB 法によつてバルサムに封入されたものとについて比較を行なつて，次の結果をえた．

その鋸歯状突起はいずれもよく保存されているが，その切断数については，差がある．すなわち凍結乾燥直後，真空中でグリセリンに包埋された細胞の葉緑体には，切断がみられないのに対してさらにA法およびB法などの処理の行なわれたものには，程度の差はあるが，切断が生じる．なかでも凍結乾燥直後グリセリンに包埋されていない状態で，真空の破られているB法の場合には，A法の場合にくらべて切断数が多いことは注目に値する．しかし，この差の起る原因については，本研究の結果からだけでは，決定的な結論を下しえない．

なお凍結乾燥後にホルマリン蒸気で固定した細胞は，形態的にも組織化学的にも，固定を行なわなかつたものにくらべて，特別の相違を認めることができなかつた．

本研究の結果によると，*Spirogyra ellipsospora* を形態学的にみて，新鮮材料に近い状態に保存するのに，凍結乾燥法がすぐれていることは認められるが，これら藻体形態の固定に関しては special chromo-acetic-osmic solution もかなりすぐれている．しかし，この固定液を用いるときは，バルサムに封入した直後の細胞容積の減少が凍結乾燥B法の場合にくらべてはげしく，葉緑体の切断数は凍結乾燥A法の場合にくらべて，はるかに多い．なおこの固定液は，オスミウムやその他の重金属などを含んでいるから，一般の組織化学的研究用の固定液としては不適当である．

上にのべたように凍結乾燥法は，形態学的研究に適した方法であるが，組織化学的研究にも適している．たとえば *Lilium longiflorum* の子房を 10% ホルマリンで固定した材料で検出できなかつた反応が，凍結乾燥法により処理した材料では検出でき，新鮮材料での反応に近い．本研究で得た benzidine 反応の結果はこの一例としてあげることができる．また，凍結乾燥をした材料や新鮮材料におけるものとくらべて，10% ホルマリンで固定した材料では，染色される範囲がせまくなつているという例もある．したがつて，凍結乾燥法はすくなくとも現在では，形態学的にも，組織化学的にも，上記の材料に関し，最もすぐれた固定法であるといえる．

要　約

1. 凍結乾燥法により，*Spirogyra ellipsospora* および *Lilium longiflorum* の子房を処理した．前者については形態学的研究を，後者については組織化学的研究を行なつた．

2. 本研究では，上記の材料を $-150°$ 以下で凍結した後，$-50°$ 附近で，減圧乾燥した．

3. 凍結乾燥法により処理し，真空中でグリセリンに包埋した *Spirogyra ellipsospora* では，細胞容積の増大がみられたが，細胞壁の破壊は少なく，葉緑体の鋸歯状突起はよく保存され，その切断もみられない．しかし，細胞を包埋しているグリセリンを水で除いた後に，染色・脱水・バルサム封入などをほどこしたものでは，しばしば葉緑体の切断がみられる．

4. *Lilium longiflorum* の組織化学的研究において，凍結乾燥法で処理した材料では，10% ホルマリンで固定した材料で検出できなかつた反応が検出できる場合，また 10% ホルマリンで固定された材料で検出できるものとくらべても，凍結乾燥法で処理した材料の方が反応が強くあらわれる場合，なお新鮮材料や凍結乾燥法で処理した材料とくらべて，10% ホルマリンで固定した材料では，染色される範囲が狭くなつている場合などがある．

5. (3)・(4) の結果から考えると，本方法は藻類の形態固定にすぐれていると考えられる．Special chromo-acetic-osmic solution や，組織化学的研究の際によく用いられるホルマリン固定液のいずれよりも，形態学的・組織化学的にすぐれた植物細胞の処理方法である．

謝　辞

以上の研究において終始われわれを，御指導下さつた新家浪雄教授に感謝いたします．

また，*Spirogyra ellipsospora* の同定を快よくひきうけて下さつた根来健一郎博士に感謝いたします．

引 用 文 献

1) Altmann, R., Die Elementarorganismen und ihre Beziehungen zur den Zellen. Leipzig (1890). 2) Gersh, I., Anat. Rec. **53**:309 (1932). 3) Mancini, R. E., Anat. Rec. **101** : 149 (1948). 4) Emmel, V. M., Anat. Rec. **95** : 159 (1946). 5) Goodspeed, T.H. and Uber, F.M., Proc. N. A. S. **20** : 495 (1934). 6) Müller, H. R., J. Ultrastructure Res. **1** : 109 (1957). 7) Patten, S.F. and Hopkins, A.L., Exp. C. Res. **14** : 647 (1958). 8) Chamberlain, C. J., Methods in plant histology. Chicago (1924). 9) Jackson, C., Onderstepoort J. Vet. Sci. Animal Ind. **19** : 169 (1944).

Summary

1. *Spirogyra ellipsospora* and ovary slices of *Lilium longiflorum* are freeze-dried. The former was used in the morphological investigation and the latter in the histochemical.

2. Materials were frozen in iso-pentane at about $-150°$ and was dried at about $-50°$ *in vacuo*.

3. In *Spirogyra ellipsospora*, the cells increased in volume when they were freeze-dried and embedded in glycerin *in vacuo*. In these cells, chromatophores were not segmented and their serrated margins were well preserved, while chromatophores were often segmented when the cells were stained, dehydrated and mounted in Canada balsam after frozen and dried. Moreover, the cell volume did not show marked changes and serrated margins of the chromatophores were well preserved.

4. In the freeze-dried ovary tissues of *Lilium longiflorum*, benzidine reaction was positive while in the tissues fixed by 10% formalin the reaction was negative. Moreover, in the freeze-dried tissues, Fehling's reaction, Sudan III reaction, and M-nadi reaction showed stronger positive reaction than the reaction in the tissues fixed by 10% formalin. Further, in the Sudan III reaction, the staining area of the fixed tissues by 10% formalin was narrower than that of freeze-dried and fresh tissues.

5. From the results stated above, it is concluded that the freeze-drying method gives more favorable results than either the fixation by special chromo-acetic-osmic solution which is generally used in morphological study of *Spirogyra*, and or the fixation by 10% formalin solution which is generally used in histochemical study of *Lilium longiflorum*.

日本植物学会会則

（昭和 31 年 7 月 14 日改正）

第1条　本会は日本植物学会という.

第2条　本会は植物学の進歩と普及をはかり，あわせて会員おたがいのしたしみを増すのを目的とする.

第3条　本会は前条の目的を達するために「植物学雑誌」そのほかの出版物の刊行，大会・講演会・講習会などの開催，そのほか必要と思われる事業を行う.

第4条　本会の会員は次の5種とする:

通常会員・終身会員・特別会員・外国通信会員・名誉会員

第5条　通常会員とは所定の会費を納めるものをいい，終身会員とは所定の終身会費を納めたものをいう.

第6条　特別会員とは引続き本会の会員であつて本会に対していちじるしい功労のあつた者，外国通信会員とは本会に関係の深い外国人，また名誉会員とは植物学に対して功労のいちじるしい者で，いづれも評議員会で協議し会長が総会で推薦し承認された者をいう．但しやむを得ない場合は，あとで総会の承認を求めることがある．これらの会員は会費を要しない.

第7条　本会には地方支部を置き，会員はいずれかの地方支部に属するものとする．地方支部についての規定は別に設ける.

第8条　本会には次の役員を置く:

会長 1 名・幹事長 1 名・幹事 若干名・評議員 若干名・編集委員 若干名

第9条　役員は会員の中から選出し，任期は2ヵ年とする．但し重任することができる．選出についての規定は別に設ける.

第10条　会長は会務の全体をすべる．幹事長は会長を助けて会務を処理する．幹事は庶務・会計・編集・図書管理など日常の会務を行う.

第11条　評議員は評議員会を構成する．評議員会は会長の諮問の範囲で本会の要務を審議し，また総会への提案を作る.

第12条　編集委員は編集委員会を構成する．幹事長はその長となる．編集委員会は「植物学雑誌」の編集に関しての一切の責任を負う.

第13条　本会の会計年度は1月に始まり12月に終る.

第14条　本会は原則として毎年1回総会を開き，会務を協議し議決する．なお会長が必要と認めた場合には臨時総会を開くことができる.

第15条　本会は総会の時大会を開き研究発表などを行う．大会には大会会長そのほか若干名の臨

（裏面へつづく）

きりとり線

入会申込書

氏　名 ふりがな	男　女 明治 大正　年　月　日生 昭和	この紙を切りとつて所要の事がらを記入または○でかこみ会費をそえて学会あてにお送り下さい．どなたでも入会できます.
勤務先 （所在地）		
住　所		
通常 終身　会員に	昭和　年　から	支部へ　雑誌の送り先を指定して下さい．希望する方へ○印を

入会の申込，会費（年 900 円の払込，そのほか会へのご連絡のあて先は：

東京都文京区東京大学理学部植物学教室内　日本植物学会です.

それから会費の払込は振替貯金口座東京 11190 番を利用されるのが最も確実です．なお振替でお払込の場合は特に領収書をさし上げませんからあしからず.

時の役員を置くことができる．大会会長は会長が推薦し，そのほかの役員は大会会長が依嘱する．

第16条　会員は会合に出席して講演をし議事に参加し，「植物学雑誌」に投稿し，また本会所有の図書を閲覧することができる．また毎号無料で「植物学雑誌」の配布を受ける．

第17条　会員が退会しようとするときは，そのことを本会に通知しなければならない．もし会費の滞納があるときはその際全額を納めなければならない．但し既に納めた会費は一切これを返さない．通常会員が会費を滞納したときは直ちに前条の権利を停止し，1カ年以上滞納したときは除名することがある．

第18条　本会の会則または付則を変更するには総会または臨時総会でこれを協議し，出席会員の3分の2以上の同意を得なければならない．

付則第1　会　　費（会則第5条関係）

第1条　通常会員の会費は年900円とし300円ずつ分納することもできる．終身会費は15,000円とする．

このほか国外在住会員に限り植物学雑誌の送料を負担する．

第2条　評議員編集委員以外の役員は在任中会費を要しない．

付則第2　地方支部　（会則第7条関係）

第1条　地方支部は原則として50名以上の会員のある地方に置き，北海道・東北・関東・北陸・中部・近畿・中国四国・九州の8とする．

第2条　会員は居住地の支部に入るのが原則であるが，事情により他の支部に属することもできる．

第3条　支部には支部長を置く．支部長は支部を代表する．

第4条　そのほかの規定は各支部ごとに設ける．

付則第3　役員の選出方法　（会則第9条関係）

第1条　会長は全会員の投票で選出される．その際評議員会は若干名の候補者を推薦することができる．

第2条　評議員は各支部別に支部会員によつて選出される．その定員は各支部最低2名とし，会員数が100名を越える支部では50名までごとに1名を増す．評議員は引続き3期選出されることはできない．なお会長および幹事長は評議員を兼任することができない．

第3条　幹事長・幹事・編集委員はいずれも会長が依嘱する．

………… ………… … … ………………… きりとり線

入会や転居などの場合には必ず別に支部へも連絡して下さい．支部のあて先は次のとおりです．なおどの支部へ入るかは大体地理的にきまるわけですが，ご都合でA支部よりもB支部の方が便利だという方はB支部に入られてもよいことになつています．

北海道支部	札幌市北8条西5丁目	北海道大学理学部植物学教室
東北支部	仙台市片平丁	東北大学理学部生物学教室
関東支部	東京都文京区大塚町	お茶の水大学理学部生物学教室
北陸支部	金沢市仙石町	金沢大学理学部植物学教室
中部支部	名古屋市千種区不老町	名古屋大学理学部生物学教室
近畿支部	京都市左京区北白川	京都大学理学部植物学教室
中国四国支部	広島市東千田町	広島大学理学部植物学教室
九州支部	福岡市箱崎	九州大学理学部生物学教室

Studies on the Light Controlling Flower Initiation of *Pharbitis Nil*. IV. Further Studies on the Light Preceding the Inductive Dark Period

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

Received July 3, 1959

Low-intensity light preceding the inductive dark period of 16 hours inhibits flower initiation in *Pharbitis* seedlings, however, the low intensity light including little far-red light inhibits only a little and that including far-red light does so heavily[1]. Flower inhibitory effect of this far-red light is reversed to some extent by a brief red light given just before the dark period[1,2]. On the other hand, low-intensity light with or without far-red light preceding a dark period of 12 hours or less, promotes flowering response remarkably[2,3].

In *Pharbitis* seedlings, a 16-hour dark period can induce maximum flowering response and further lengthening of the dark period hardly increases the responses, but a 12-hour dark period is not so inductive as to bring about a maximum flowering response, and further lengthening of the dark period increases flowering response greatly. It was supposed that the first process of the inductive dark period proceeds under low-intensity light or far-red light as well as in darkness, and that the far-red light prevents the subsequent dark process[2,3].

Far-red light preceding a dark period of 12 hours or less promotes flowering responses. If brief red light is given between the far-red light and the dark period, there is a question whether the flowering response increases or not. Considering that the red light can reverse the flower-inhibitory effect of the far-red light, a greater increase in flowering response might be expected. On the contrary, it is also conceivable that the red light following the far-red light may have a "light-break" effect, i.e. a flower inhibitory effect. The light break is known to be most effective in the first hours of a long dark period[4,5,6,7], and the first process of the inductive dark period presumably is undisturbed by far-red light [2,3].

Low-intensity light preceding a 16-hour dark period inhibits flower initiation to some extent even if it contains little far-red light[1,2]. If the brief red light is given between the low-intensity light and the dark period, the inhibitory effect of low-intensity light may or may not be reversed. The first process of the inductive dark period is considered to proceed under such a low-intensity light. It is also possible that the brief red light following the low-intensity light has a light-break effect, and inhibits the flowering response.

The effect of a brief red light inserted between the inductive dark period and the preceding low-intensity light, far-red light or darkness was investigated here.

Material and Methods

Material used was the seedling of *Pharbitis Nil*, strain "Violet". Methods of experiments were similar to those described in a previous paper[1]. Light filters and light sources used were also described there.

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

Experiments and Results

Experiment 1. Plants were placed in darkness for 2, 4 and 8 hours, at the end of which time they were exposed to red light (600–700 mμ) of 3000 erg/cm.^{2}/sec. for 5 minutes, which inhibits flower initiation when given at the middle of a 16-hour dark period. Subsequently one group of the plants (Group 1) was subjected to 16 hours of darkness, which is enough to induce a maximum flowering response, and the other group (Group 2) to 12 hours of darkness, which is not so inductive as to bring about a maximum flowering response. Control plants were subjected to 16- and 12-hour darkness preceded by sun light. Results are shown in Table 1.

Table 1. Flowering response of plants which were exposed to 2 to 8 hours of darkness, 5 minutes of red light of 3000 erg/cm.^{2}/sec., and 12 or 16 hours of darkness, successively.
(Treated on May 1 and dissected on May 15, 1958)

Group	Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
1	16^hd	38	100	5.0	100
	2^hd→5′R→16^hd	38	100	4.8	100
	4^hd→ 〃 → 〃	39	100	4.9	100
	8^hd→ 〃 → 〃	32	87.5	1.8	0
	24^hd	36	100	5.2	97.2
2	12^hd	38	100	1.1	0
	2^hd→5′R→12^hd	38	100	4.5	94.7
	4^hd→ 〃 → 〃	39	100	4.8	100
	8^hd→ 〃 → 〃	36	25.0	0.4	0
	20^hd	40	100	4.9	97.5

2^hd→5′R→16^hd: 2-hour darkness, 5 minutes of red light and 16-hour darkness were given successively.
These notations will be used hereafter.

In Group 1, 2- or 4-hour darkness preceding 5 minutes of the red light had little effect on flowering response, but with 8 hours of darkness preceding the red light, flower initiation was heavily inhibited. This is very similar to the results obtained with *Xanthium*[5,6]), in which the light interruption does not inhibit flowering at the 2nd to 4th hour of the long dark period but inhibits it strongly at the 6th to 8th hour.

In Group 2, when a 12-hour dark period was preceded by another dark period of 2 or 4 hours duration, the two dark periods being separated by 5 minutes of red light, flowering response was promoted remarkably, but when preceded by 8 hours of darkness and 5 minutes of red light, it was inhibited. A 12-hour dark period can not induce maximum flowering response, and further lengthening of dark period increases flowering response strikingly; therefore, if 2 or 4 hours of darkness precede the 12-hour darkness, even if the red light was inserted between them, flowering response is increased, for the flower-inducing dark process is stable to the brief light during the first 4 hours of the dark period. After 8 hours of darkness, the flower-inducing dark process becomes light-sensitive; therefore, flowering response is reduced strikingly if an 8-hour dark period precedes the 12-hour dark period with intervening red light.

Experiment 2. Two groups of the plants were exposed to daylight fluorescent light of 50 erg/cm.²/sec. (FL) containing little far-red light. The exposure lasted 8 hours preceding a 6- to 16-hour dark period, and one group was subjected to red light for 5 minutes just before the dark period, while the other one was not. Control plants were given 6 to 16 hours of darkness preceded by sun light. Results are shown in Table 2.

Table 2. Flowering response of plants which were exposed to 8 hours of daylight fluorescent light of 50 erg/cm.²/sec., 5 minutes of red light of 3000 erg/cm.²/sec. and a dark period of various hours, successively.
FL: Daylight fluorescent light of 50 erg/cm.²/sec.
(Treated on May 26 and dissected on June 8, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
$8^hFL \rightarrow 5'R \rightarrow 16^hd$	40	82.5	1.1	0
〃 → 〃 → 14^hd	40	2.5	0.0	0
〃 → 〃 → 12^hd	40	0	0	0
〃 → 〃 → 10^hd	40	0	0	0
〃 → 〃 → 8^hd	38	0	0	0
〃 → 〃 → 6^hd	40	0	0	0
$8^hFL \longrightarrow 16^hd$	39	100	4.5	94.9
〃 ⟶ 14^hd	39	100	4.9	100
〃 ⟶ 12^hd	40	100	3.9	67.5
〃 ⟶ 10^hd	39	100	4.4	74.4
〃 ⟶ 8^hd	40	100	2.6	17.5
〃 ⟶ 6^hd	39	61.6	0.7	0
16^hd	39	100	4.3	100
14^hd	39	100	1.9	2.6
12^hd	39	5.1	0.1	0
10^hd	40	0	0	0
8^hd	40	0	0	0
6^hd	40	0	0	0

Eight-hour FL did not inhibit the flowering when followed by a 16-hour dark period, and promoted remarkably when followed by a dark period of 14 hours or less. In the previous papers[1,2]), it was reported that the 8-hour FL preceding 16 hours of darkness inhibited flowering to some extent. In the present experiment, plants were very sensitive to photoperiodic induction and in such a case the flower inhibitory effect of the FL may become obscure. In another experiment which is not presented here, the plants were not so sensitive and the flower inhibitory effect of FL was more obvious*.

If 5 minutes of red light was given following the 8-hour FL (preceding dark period), flowering response was reduced remarkably, irrespective of the duration of darkness. This suggests that, after the 8-hour FL, under which the first process of the inductive dark period proceeds, the reactions inducing flower initiation become light-sensitive, probably producing some light-sensitive substance which is destroyed by 5 minutes of red light. The same may also be the case in complete darkness (cf. Experiment 1).

Experiment 3. Plants were treated in the same way as in Experiment 2, but

* Photoperiodic sensitivity of *Pharbitis* seedlings varies with the seasons, being influenced by unknown internal and external factors. From spring to early summer, the sensitivity for photoperiodic induction is the highest.

far-red light (700–100 mμ) of 120 erg/cm.^{2}/sec. was mixed simultaneously with the FL of 8 hours. As has been reported previously, under this light the first process of the inductive dark period is believed to proceed, but this light inhibits flower initiation by preventing the subsequent dark processes[2,3]. Table 3 shows the results.

Table 3. Flowering response of plants which were exposed to 8 hours of FL+FR, 5 minutes of red light of 3000 erg/cm.^{2}/sec. and a dark period of various hours, successively.
FL+FR: Daylight fluorescent light of 50 erg/cm.^{2}/sec. mixed with far-red light of 120 erg/cm.^{2}/sec.
(Treated on May 27 and dissected on June 11, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
8^h(FL+FR)→5′R→16^hd	39	94.9	2.3	7.7
〃 →〃 →14^hd	40	2.5	0.0	0
〃 →〃 →12^hd	39	0	0	0
〃 →〃 →10^hd	38	0	0	0
〃 →〃 → 8^hd	40	0	0	0
〃 →〃 → 6^hd	40	0	0	0
8^h(FL+FR) ⟶ 16^hd	30	61.5	0.6	0
〃 ⟶ 14^hd	40	70.0	0.8	0
〃 ⟶ 12^hd	38	36.9	0.4	0
〃 ⟶ 10^hd	40	37.5	0.4	0
〃 ⟶ 8^hd	40	42.5	0.5	0
〃 ⟶ 6^hd	36	0	0	0
16^hd	43	100	4.3	100
14^hd	40	100	1.9	2.5
12^hd	40	32.5	0.3	0
10^hd	39	0	0	0
8^hd	39	0	0	0
6^hd	39	0	0	0

When a 16-hour dark period was preceded by 8-hour FL mixed with far-red light (FL+FR), flower initiation was inhibited, but this inhibition was reversed to some extent by the red light of 5 minutes given just before the dark period. This reversing effect of red light was reported previously[1,2]. The first process of the inductive dark period proceeds under the FL+FR, and red light has a light-break effect which tends to counteract this process and thereby inhibits flowering. In the present case, however, the reversing effect of red light for the flower-inhibitory effect of far-red light evidently exceeds the light-break effect, and the net result is some stimulation of flowering. When the dark period is 12 hours or less, 8-hour FL+FR preceding the dark period promoted flower initiation to some extent, and if the red light of 5 minutes was given just before the dark period, flowering response was inhibited. Flower inhibitory effect of the red light, i.e. the light-break effect, is believed to exceed the reversing effect, in this case.

Experiment 4. Plants of the first group were subjected to darkness and those of the second group to FL for 2, 4, 6 and 8 hours, respectively. Subsequently both groups were exposed to red light of 3000 erg/cm.^{2}/sec. for 5 minutes, and thereafter to 12 hours of darkness. Plants of the third group (control plants) were subjected to 8-, 12-, 14-, 16- and 20-hour dark periods preceded by sun light. Results are shown in Table 4. Flowering responses of the first group are very similar to those of the plants in Experiment 1. A light break at the 2nd to 6th hour of the dark period is

Table 4. Flowering response of plants which were exposed to darkness or FL of 2–8 hours, 5 minutes of red light of 3000 erg/cm.²/sec. (R) and a 12-hour dark period, successively.
FL: Daylight fluorescent light of 50 erg/cm.²/sec.
(Treated on June 12 and dissected on June 26, 1958)

Group	Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
1	$2^hd \to 5'R \to 12^hd$	37	100	3.6	13.5
	$4^hd \to$ 〃 $\to$ 〃	38	100	4.1	29.0
	$6^hd \to$ 〃 $\to$ 〃	37	94.6	3.6	21.6
	$8^hd \to$ 〃 $\to$ 〃	38	0	0	0
2	$2^hFL \to 5'R \to 12^hd$	38	100	3.5	31.6
	$4^hFL \to$ 〃 $\to$	39	100	4.3	84.7
	$6^hFL \to$ 〃 $\to$	40	95.0	2.5	7.5
	$8^hFL \to$ 〃 $\to$	39	0	0	0
3	8^hd	40	0	0	0
	12^hd	38	84.3	1.6	0
	14^hd	38	100	4.0	13.2
	16^hd	39	100	4.7	84.7
	20^hd	40	97.5	3.5	27.5

less effective, but that at the 8th hour inhibited flowering response completely.

The FL and darkness have exactly the same effect when followed by 5 minutes of red light and subsequent 12-hour dark period. This supports again the hypothesis that the first process of the inductive dark period proceeds under FL as easily as under complete darkness[2,3].

Experiment 5. The same experiment as Experiment 4 was performed, but the far-red light of 120 erg/cm.²/sec. was mixed with the FL (FL+FR). Results are shown in Table 5.

In this experiment, too, the flowering response of the first and the second group

Table 5. Flowering response of plants which were exposed to darkness or FL+FR of 2–8 hours, 5 minutes of red light of 3000 erg/cm.²/sec., and a 12-hour dark period, successively.
FL+FR: Daylight fluorescent light of 50 erg/cm.²/sec. mixed with far-red light of 120 erg/cm.²/sec.
(Treated on May 26 and dissected on June 8, 1958)

Group	Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
1	$2^hd \to 5'R \to 12^hd$	40	100	2.0	2.5
	$4^hd \to$ 〃 $\to$ 〃	40	100	4.0	47.5
	$6^hd \to$ 〃 $\to$ 〃	39	100	3.7	43.6
	$8^hd \to$ 〃 $\to$ 〃	40	52.5	0.7	0.
2	$2^h(FL+FR) \to 5'R \to 12^hd$	40	97.5	2.7	2.5
	$4^h(FL+FR) \to$ 〃 $\to$ 〃	40	105	4.6	95.0
	$6^h(FL+FR) \to$ 〃 $\to$ 〃	40	97.5	2.9	20.0
	$8^h(FL+FR) \to$ 〃 $\to$ 〃	39	2.6	0.0	0
3	12^hd	40	72.5	0.9	0.
	14^hd	40	100	2.8	2.5
	16^hd	38	100	4.2	57.9
	20^hd	39	97.4	4.5	74.4

showed no remarkable difference. This implies that the first process of the inductive dark period can proceed under FL+FR as easily as under FL or darkness, and that the flower inhibitory effect of far-red light which exerts an influence upon the following dark process is reversed by 5 minutes of red light; thus, the results become similar to those obtained in Experiment 4.

Only the plants exposed to the red light following an 8-hour FL+FR initiated considerably fewer flower primordia than those darkened for 8 hours preceding the red light, probably owing to insufficient reversing effect of the red light for the flower inhibitory effect of 8-hour FL+FR. It was reported in a previous paper, that far-red light of 2~4 hours preceding the inductive dark period is not so effective for flower inhibition but that of 8 hours is very effective[2].

Discussion and Conclusions

From the results mentioned above, it is concluded as follows:

The first process of the inductive dark period proceeds under FL, FL+FR and darkness, and this process is stable to red light of 5 minutes (3000 erg/cm.^{2}/sec.). But the process taking place 6–8 hours after the beginning of the first process is light-sensitive and destroyed or nullified by the brief red light. Probably, a light-sensitive substance, which is destroyed by the red light, is produced after 6–8 hours of FL, FL+FR or darkness.

As has been reported previously, far-red light preceding the inductive dark period has a dual effect, i.e. flower-promoting and flower-inhibitory effect[2,3]. The former is attributable to the fact that the first process of the inductive dark period can proceed under far-red light, and the latter to the effect on the " red-far-red absorbing pigment system ", for the flower-inhibitory effect of far-red light is reversed by red light to some extent.

Red light following the far-red light is also considered to have a dual effect, i.e. a light-break effect and a reversing effect on the " red-far-red absorbing pigment system ". The former is flower inhibitory and the latter is flower promoting. The observable effect of the red light following far-red light depends on which of these two effects is the stronger.

Summary

1) Five minutes of red light of 3000 erg/cm.^{2}/sec. given at the 2nd to 4th hour of a long dark period does not inhibit flower initiation, but that given at the 8th hour does so.

2) Eight-hour daylight fluorescent light of 10 lux (FL) preceding the dark period inhibits flower initiation a little when the dark period is 16 hours or more, but promotes when the dark period is 12 hours or less. Red light of 5 minutes following the 8-hour FL inhibits flowering response strongly irrespective of the duration of darkness. Probably, the red light has a light-break effect, because the first process of the inductive dark period is considered to proceed under the FL.

3) Eight-hour FL mixed with far-red light of 120 erg/cm.^{2}/sec. (FL+FR) preceding the dark period inhibits flower initiation if the dark period is 14–16 hours, but promotes if the dark period is 10 hours or less. The red light of 5 minutes inserted between them reduced the flowering responses when followed by a dark period of 14 hours or less, but increased flowering to some extent when followed by a 16-hour dark period.

4) Red light following the FL+FR is considered to have a dual effect:

i) It reverses the flower inhibitory effect of FL+FR preceding the inductive dark period.

ii) It has a light-break effect, i.e. a flower inhibitory effect, through counteracting the first process of the inductive dark period, a process which presumably proceeds under FL+FR.

The observable effect of red light following FL+FR depends on which effect is the stronger.

5) FL, FL+FR and darkness have almost the same effect on flower initiation when given for 2–6 hours preceding a 12-hour dark period, with 5 minutes of red light intervening.

Grateful acknowledgement is given to Professor S. Imamura for his suggestion and criticisms.

References

1) Takimoto, A. and Ikeda, K., Bot. Mag. Tokyo, **72**: 137 (1959). 2) —— and ——, ibid. **72**: 181 (1959). 3) —— and ——, ibid. **72**: 388 (1959). 4) Carr, D. J., Physiol. Plantarum, **5**: 70 (1952). 5) Salisbury, F. B., Plant Physiol., **33**: Supp. 24 (1958). 6) —— and Bonner, J., Plant Physiol., **31**: 141 (1956). 7) Wareing, P. F., Physiol. Plantarum, **7**: 157 (1954).

摘　　要

アサガオの花芽形成を支配する光条件について.
IV. 暗期前の光について. 続報

滝 本　敦・池 田 勝 彦

1) 長い暗期 (16 時間以上) の最初の 2〜4 時間目に 5 分間 3000 erg/cm.²/sec. の赤色光を与えても花芽形成は抑制されない. しかし 8 時間目に与えるといちじるしく抑制される.

2) 暗期前 8 時間 10 ルックスの昼光色螢光燈光 (FL) を与えると, 暗期が 16 時間以上の場合には, わずかに花芽形成が抑制され, 暗期が 12 時間以下の場合には花芽形成が促進される. 8 時間 FL 照射後, 5 分間赤色光を与えると暗期の長さに関係なく花芽形成は抑制される.

暗期反応の初期段階は FL 下で進行し得るものと考えられるので, この赤色光の効果は恐らく光中断効果によるものであろう.

3) FL に 120 erg/cm.²/sec. の近赤外光を混ぜた光 (FL+FR) を暗期前 8 時間与えると, 暗期が 14 時間以上の場合には花芽形成が抑制され, 暗期が 10 時間以下の場合には促進される.

FL+FR と暗期の間に与えた 5 分間の赤色光は暗期が 16 時間以上の時には花芽形成を促進し, 暗期が 14 時間以下の場合には花芽形成を抑制する.

4) FL+FR 照射後に与えた赤色光は二重の効果を有するものと考えられる.

i) 暗期前に与えた FL+FR の花芽形成抑制効果を消却する.

ii) 暗期反応の初期段階は FL+FR 下で進行し得ると考えられるので, この赤色光は光中断効果 (花芽形成抑制効果) を有する.

FL+FR 後に与えた赤色光の効果としては, 上記二重効果の差が現われてくる.

5) 12 時間の暗期前に 2〜6時間, FL, FL+FR または暗期を与え, 両者の間に 5 分間の赤色光を与えると, 開花反応はいずれの場合もほとんど同じである. すなわち, 暗期反応の最初の 2〜6 時間は, FL, FL+FR 下で暗黒中におけると同様に進行しているものと考えられる. (京都大学農学部応用植物学研究室)

On the Life Cycle of *Spirillum japonicum*

by Narumi WATANABE*

Received June 2, 1959

Since the life cycle of *Azotobacter* was studied by Löhnis and Smith[1]), many investigations mainly on those of bacilli have been carried out, but no reports on *Spirillum* except the recent ones of Williams's[2,3]). The following paper by the author is an attempt to describe the results of the study of the life cycle of halophilic *Spirillum japonicum*[4]) in a liquid medium.

Method

Spirillum japonicum grows well both on medium No. 1 and No. 2 at 22°. The cells obtained by liquid culture were mainly stained by the vital-staining method and observed under the microscope. Löffler's methylene blue solution diluted twice to thrice was used for staining and found very effective. *Spirillum* can live and keep moving in the dye for some time after it is stained. The method of flagellum-staining after Sugahara and Nishizawa adopted in this experiment proved successful. Carbol fuchsin solution as second solution is highly efficient to examine the minute structure. In this experiment, Ziehl's carbol fuchsin solution was sometimes used besides methylene blue solution, but it did not indicate the inner structure. Even the electron microscopic observation gave no satisfactory results. The reasons may be: 1). The cells of bacteria are so large that their contents immediately shrink due to desiccation in the vacuum-room. 2). The cell is too thick for the electron microscope to show the inner structure.

Results

1. *Formation of conjunction capsule*

In fresh culture the bacteria are very motile and reveal their typical spiral forms. The spiral cells divide through transverse fission like other bacteria. Young vegetative cells possess protoplasm which can be stained homogeneously by methylene blue solution, with fine unstained granules running obliquely to the long axis. These fine granules are not stained even by carbol fuchsin solution, so it may be assumed that they are not protoplasm but part of the cell membrane (Fig. 1. ×1350).

The aerobic nature of this kind of bacteria brings about a turbid layer of about 1–1.5 cm. thick under the surface of the medium. In 40–48 hours culture fission becomes less active and stained granules are seen in the cell. At this stage each organism located on the surface of the medium is observed growing a capsule at the end like a cap. It may be appropriate to call the capsule " conjunction capsule " (Fig. 2. ×1100). As the flagella grow out of the protoplasm, the dot observed on the flagella and located a little distance away from the pole is assumed to be the crossing point of the capsule and the flagella (Fig. 3. ×1350). As soon as a capsule begins to form, the spiral movement becomes slow while the cell starts vibrating.

2. *Radiate conjunction*

The cell with a capsule gathers each other and conjoin at the capsule, and a

* Biological Institute, Faculty of Education, Chiba University, Chiba, Japan.

number of cells thus united take a radiate form (Fig. 4a & 4b. ×1350). The number of uniting organisms gradually increases up to about forty. An unstained space is noticed where these cells join each other, which may indicate the existence of some capsules that can not stained by the flagellum-staining. Considering that the flagella remain unchanged in the center of the cells which are closely united at the poles as the conjunctive action proceeds, it is assumed that flagella take no part in the radiate arrangement (Fig. 5. shown by flagellum-staining. ×1350). Then a number of radiate forms are observed macroscopically to gather into a large mass floating like lumps of cloud in the medium (Fig. 6. ×550), and sink down to the bottom by slight stimulus.

Then the adhesive power of the organisms by which the radiate arrangement is maintained is so strong that they do not separate when artificially stimulated, for instance, by a stream of water passing between the deck glass and the slide glass or by a pressure given over the deck glass (Fig. 7. ×550). The radiate arrangement is preserved for a long time in the preparation fixed with formalin (Fig. 8. stained with carbol fuchsin solution after fixation. ×1100).

One giant organism about twice as thick as the normal one is always attached to the center of a radiate form. (Fig. 8, Fig. 9a & 9b. ×1350) Later this giant cell comes to contain some large stained grains which may derive from the protoplasmic substance (Fig. 10. ×1350). The giant cell, which the writer would like to name " stalk cell " provisionally, is supposed to keep the radiate form stable in the medium.

The central part of the radiate form is too intensely stained to admit a glimpse into its structure (Fig. 9). Even the electron microscopic observation does not reveal the details of the radiate arrangement. Therefore, we are obliged to define the significance of the radiate arrangement by its subsequent behaviors. At the anaphase of the radiate arrangement, every one of the organisms except the giant one becomes thin while all the protoplasm in every cell transfers to the middle of the cell. Thus both the stained and unstained parts are seen in the same cell (Fig. 11. ×550).

3. *Exocyst formation*

The radiate arrangement becomes looser and looser and finally it breaks up into free organisms making a slow spiral movement. A protuberance is made on the lateral side of the middle part of some organisms (Fig. 12. ×1350). The incipient protuberance membrane is so fragile as to release the contents by slight stimulus. The contents released from the cell are the protoplasm containing the stained granules. After the release, the mother cell becomes empty (Fig. 13. ×1350).

As the globular protuberance grows bigger, it absorbs the greater part of the protoplasm and the stainable granules in the mother cell. (Fig. 14a & 14b. ×1350) When the exocyst is formed, the organism sinks to the bottom of the medium and then the exocyst is separated from its mother cell. All these processes need 5–6 days to complete after the inoculation. After the completion of the exocyst, the mother cell takes a loose spiral form containing several stained grains arranged in a row. Each stained grains consists of several stained granules (Fig. 15. ×1100). When an organism becomes extinct or destroyed, the grains are scattered in the medium and disappear (Fig. 16a & 16b. ×1350). The stalk cell still retains its giant shape after the loosening of the radiate arrangement and its future course is unknown.

4. *Endocyst formation*

Apart from the bacterial cells arranged in a radius, there is a group of very motile cells in the turbid layer of the medium. Often these cells are each shaped

like one wave as the result of fission. The middle part of the cell swells up and most of protoplasm migrates to this part, that is, it takes as a whole a somewhat twisted "V" or "J" shape (Fig. 17a & 17b. ×1350). The protoplasm of the cell then works to make a globular endocyst. At this time a very small portion of the protoplasm remains at both extremities, or the basal granules of the flagella. The flagellum is found firmly sticking to the cell even after the organism has lost its power to move and comes down to lie still at the bottom of the medium (Fig. 18. shown by flagellum-staining). A full-grown endocyst is often discovered remaining in the mother cell (Fig. 19. ×1350). The absence of a reflective membrane distinguishes the endocyst from the endospore of other bacteria. Compared with the exocyst, the endocyst is larger and reveals the details of its inner structure, when it is stained.

5. *Germination of cysts*

The protoplasm is reorganized in both exocyst and endocyst before germination. It is homogeneous at first, but as the time of germination approaches, it begins to form a spiral structure (Fig. 20. ×1350). When inoculated in the medium, the exocyst develops into an oval shape in four to five hours and the germ tube begins to emerge from one of the poles (Fig. 21. ×1350). And then it develops into a normal spiral shape. The mature endocyst separates itself from the mother cell and begins to germinate in the medium like the exocyst. The mother cell is left empty (Fig. 22. ×1350).

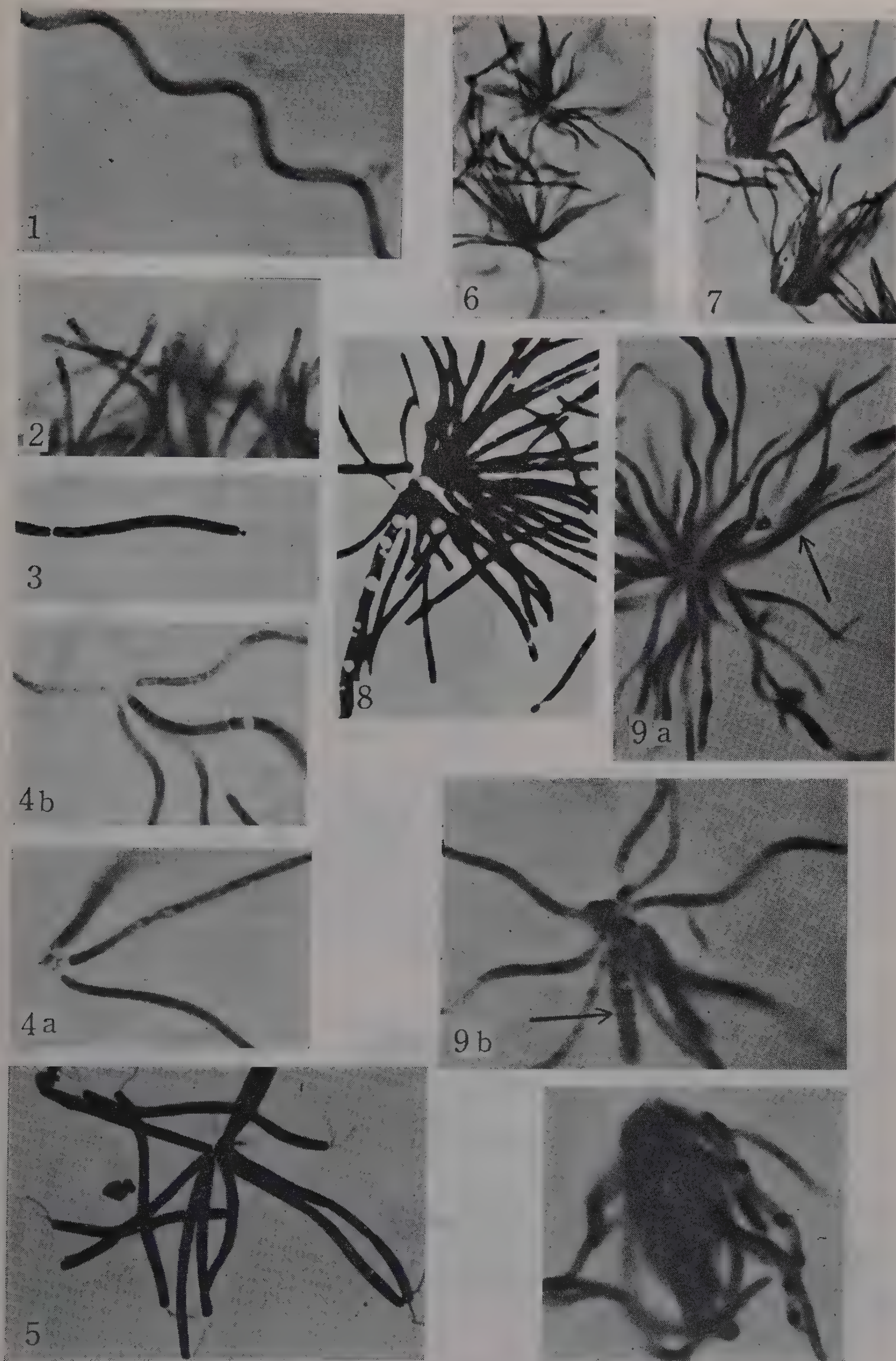
Discussion

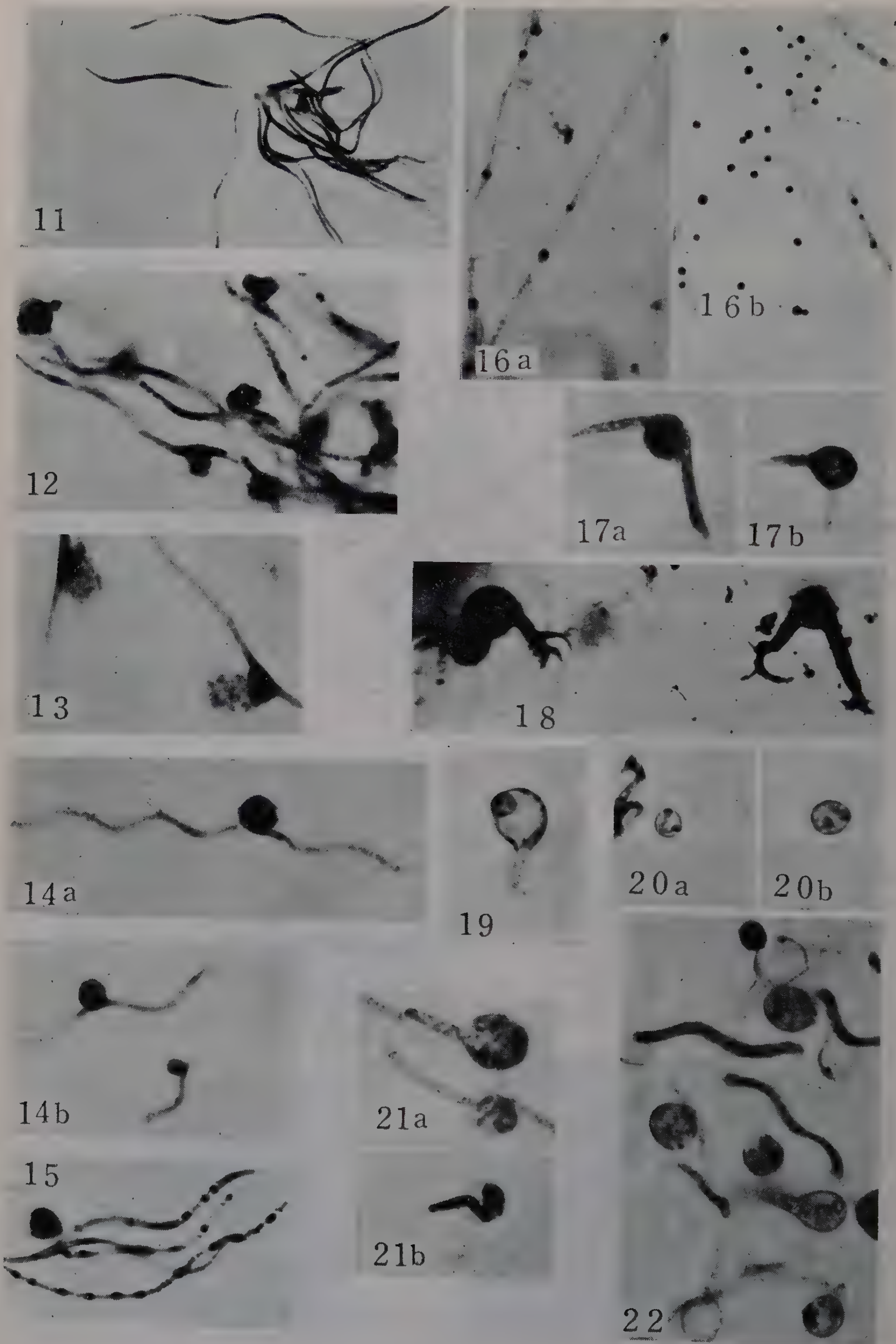
Kishitani and Sumiyoshi[5]) found the radiate form in purple bacteria and considered this phenomenon as the intermediate form between the conjugation and symplasma asserted by Löhnis. Watanabe[6]) also found the same phenomenon in *Rhodobacillus palustris*. Smith[7]) who advanced the theory of the conjugation of bacteria, reported that two bacilli make a pair end to end in *Bacteroides fundulformis*. Lederberg[8]) observed the conjugal pairing in *Escherichia coli*. Williams[2]) observed the fusion of two spirilla in *Spirillum lunatum* and stated as follows. "The establishment of sexual fusion in *Escherichia coli* by Lederberg in 1947, based on genetical studies of the exchange of character in mutants of this organism, has not been confirmed by morphological evidence of conjugation or cellular fusion in the bacteria". Apart from microscopical observation, Lederberg[9]) and Holloway[10]) reported the genetic re-combination in detail: the former on *Escherichia coli* and the latter on *Pseudomonas aeruginosa*. Lederberg[11]) referred also to the sex compatibility.

Potthoff[12]) maintained the presence of a conjugation process formed on the lateral wall in the fusion of *Rhodospirillum photometricum* and named it the "bridge." Watanabe[6]) also insisted on the presence of a cojugating capsule at the cell end of *Rhodobacillus palustris*.

Dimitroff[13]) named the equivalent of a reproductive body in *Spirillum virginianum* "coccoid body". Cayton and Preston[14]) took a photograph of the globular shape of *Spirillum manucuniense*. The reproductive body, or the "large body," which is formed as the lateral or terminal bud in *Proteus vulgaris* was reported by Stempen and Hutchinson[15,16]). Williams held the similar view of *Spirillum* and she named the body formed on the point of fusion "microcyst", from which vegetative cell germinates.

Now, the writer wants to discuss the problem of the cellular fusion on which Williams reported. She asserted that two spirilla entwined with each other to fuse.


1
2
3
4b
4a
5
6
7
8
9a
9b


11
12
13
14a
14b
15
16a
16b
17a
17b
18
19
20a
20b
21a
21b
22

But, it is obvious that an " entwined shape " can not be observed, if two spirilla are arranged in exact parallel. An entwined shape can be only observed when one spiral cell enters the other so that the two cells having the same convolution may entwine each other. And separation is only possible by going convoluting forward or backward. Therefore, it is maintained that the cellular fusion is difficult to be effected only by the attraction of a motile organism to the motionless one. Moreover, it follows that the fusion must be made at every spiral wave of the two entwined spirilla. Consequently, it is very doubtful that there is only one point of fusion between the two entwined organisms. Williams's statement as to the number of fusion points and of microcyst is very vague.

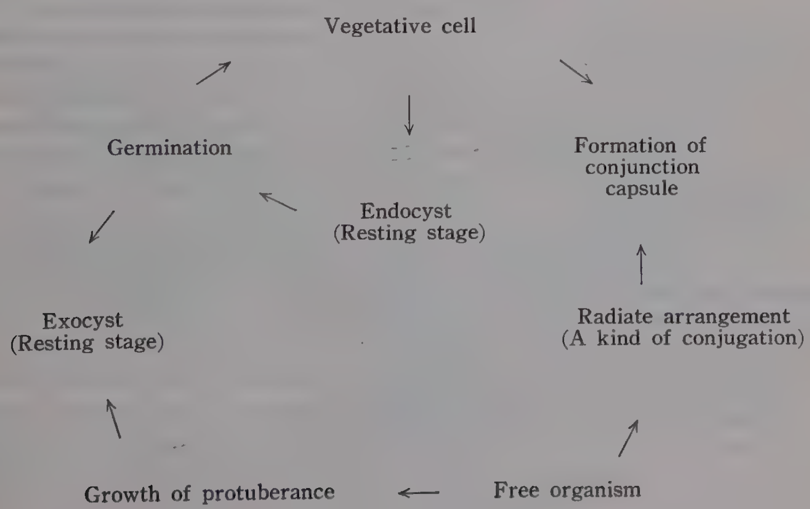
According to the writer's observation, it is not clear whether or not the migration of the protoplasm in *Spirillum japonicum* takes place between the cells at the time of the radiate arrangement. But the facts that the radiate arrangement is very stable and that the exocyst is formed with part of the protoplasm may indicate the presence of a sort of conjugation among the spirilla.

Williams[3]) observed some chromatin granules arranged in a row in the bacterial cells of *Spirillum sinosum* and *Sp. anulus*. Yuasa and Tanaka reported the presence of some nuclear materials in a granular state in young *Spirillum* sp. and in the state of some longitudinal rods at its later stage of development. The grains which appear only in the vegetative cells of *Spirillum japonicum* after the exocyst formation have a shape very similar to that nuclear substance of different dominations given by those investigators cited above. Judging from its behaviors, however, the writer has some doubts in admitting any of them as the true nuclear substance.

Summary

1. When *Sprillum japonicum* is cultured in a medium, it has two kinds of vegetative cells: one type has many conspicuous undulations and forms an exocyst, the other is shorter in length and looks like a rod-bacteria having a slight undulation and forms an endocyst.

2. The organisms of the first type with " conjunction capsule " growing at the extremities form a radiate arrangement. This phenomenon is the cellular fusion which should be assumed as a kind of conjugation.



3. The protuberance absorbing the protoplasm of each fused cell develops into a globular exocyst. Both the endocyst and the exocyst are deposited motionless in the medium as precipitate. The protoplasm in resting stage in the cyst displays itself as the spiral structure before germination. A new organism grows out of the cyst as the unipolar germination.

4. The life cycle of *Spirillum japonicum* is indicated in the diagram.

References

1) Löhnis, F. and Smith, N. R., Journ. Agr. Res., **6**: 675 (1916). 2) Williams, M. A. and Rittenberg, S. C., J. Gen. Microbiol. **15**: 205 (1956). 3) Williams, M. A., Nuclear phenomena in *Spirillum* species. (1957) (Unpublished, a dessertation presented to the University of Southern California) 4) Watanabe, N., Bot. Mag. Tokyo **72**: 77 (1959). 5) Kishitani, T. and Sumiyoshi, T., Jour. Scie. Hiroshima Univ. Series B, Div. 2. **3**: 1 (1939). 6) Watanabe, N., Bull. Fac., Education, Chiba Univ. **1**: 1 (1952). 7) Smith, W. E., Jour. Bact. **47**: 417 (1944). 8) Lederberg, J., Jour. Bact. **71**: 497 (1956). 9) ——, Genetics **32**: 505 (1947). 10) Hollway, B. W., J. Gen. Microbiol. **13**: 572 (1955). 11) Lederberg, J., Genetics **37**: 720 (1952). 12) Potthoff, H., Centr. f. Bakt. 2 Abt. **55**: 9 (1922). 13) Dimitroff, V. T., Jour. Bact. **12**: 19 (1926). 14) Cayton, H. R. and Preston, N. W., J. Gen. Microbiol. **12**: 519 (1955). 15) Stempen, H. and Hutchinson, W. G., Jour. Bact. **61**: 321 (1951). 16) ——, 1954. Sex in bacteria. Evidence from morphology. In sex in microorganism. Washington D. C. American Association for the Advancement of Science. (Cited in Williams's report.) 17) Yuasa, A. and Tanaka, K., Sci. Papers, College of General Education, Univ. Tokyo 8: 175 (1958).

摘　　要

Spirillum japonicum の生活環について

渡　辺　成　美

液体培地上に培養した好塩性螺旋菌の一種 *Spirillum japonicum* の生活環を観察した．この目的にはレフラー氏メチレン青液の 2～3 倍稀釈液を使用し，生体染色に良好な結果を得た．観察の結果栄養細胞には，形態的に二型が存在することが明らかになつた．すなわち，第一型は多数螺旋形で一種の接合と見做される繁殖法を行なうものであり，第二型はやや長桿状の単螺旋形で無性的繁殖を行なうものである．

前者の繁殖法は，いわゆる放射接着であり，二分法の減退した螺旋菌多数が集合し，菌の一端をもつて放射状に配列して強固に接着する．この際各菌端に生じた接着膜嚢が重大な役割をなすものとみられ，菌端にある鞭毛は何等の関与を示さない．接着により菌端間に原形質の移動が生ずるか否か形態的には不明であるが，それ以後の菌の行動から，一種の接合現象であると推察される．放射配列から解放された菌体には，内部原形質の中央部移動に伴ない菌側に隆起が生ずる．この隆起は母体の原形質を吸収し外生包嚢に生長する．外生包嚢は完成後，培養基中に沈澱物となり，静止期に入り，耐久性を獲得する．

後者，すなわち無性繁殖型は栄養細胞の原形質の濃縮によつて内生包嚢の形成のみを行なう．運動性を失なつた母体内から内生包嚢は完成後脱出する．内生包嚢は良好な染色性や形態から，明らかに他の細菌群にみられる内生芽胞とは区別することができる．

内生，外生包嚢とも均質染色性の原形質を有するが，その再編制により，原形質は包嚢内において螺旋形構造を示すようになる．新培地に接種された包嚢は発芽を行ない新個体に生育する．以上の観察事実より，*Spirillum japonicum* の生活環に有性，無性の繁殖法が並行する可能性を主張した．（千葉大学　教育学部　生物学教室）

Developmental Mechanics of Fucaceous Algae XIV. Plasmolysis Pattern in *Coccophora* Eggs.

by Singo NAKAZAWA*

Received July 20, 1959

Results of experiments on the plasmolysis of fucoid eggs were reported by NAKAZAWA[1]). According to him, the permeability of NaCl and saccharose generally decreases after fertilization, and plasmolysis is much more liable to take place at or near the pole where the rhizoids are to be formed than in other regions. These facts seem to be related to the phenomena that the vital staining appears more promptly after fertilization with various dyes and that it begins at or near the rhizoid pole. The present writer repeated similar experiments on *Coccophora* eggs and, as a result, could obtain some new observations especially as to the regional change in the physical properties of the cytoplasm occurring with development after fertilization.

The material was *Coccophora Langsdorfii*, collected at Asamushi in April, 1959. Eggs liberated in glass vessels were fertilized artificially, and tested at five developmental stages: (a) after liberation but before fertilization (Fig. 2A), (b) transformation stage (Fig. 2B), (c) just after the first nuclear division but before occurrence of segmentation (Fig. 2C), (d) just after the first segmentation (Fig. 2D), and (e) just before occurrence of the second nuclear division (Fig. 2E). Eggs at each stage were immersed in hypertonic solutions of NaCl and of saccharose at various concentrations. The plasmolysis pattern which occurred was inspected.

Results with Discussion

Before fertilization, the egg undergoes not plasmolysis but plasmorrhysis in hypertonic solutions. That is, the egg surface becomes irregularly wrinkled, showing that the rigid cell membrane is not formed yet. This point agrees with the results of observations in *Hormosira* by Levring[2]), and with the opinion presented by Nakazawa[3]) based on experiments of blister formation in some fucoid eggs. After fertilization, plasmolysis occurs in hypertonic solutions, and the limit concentration becomes lowered (Fig. 1), caused probably by the development of a cell membrane which is less permeable for NaCl and for saccharose. This is also in accordance with former observations[2,3]). Whitaker's unique experiment[4]) obtaining elongated *Fucus* eggs by sucking eggs into a glass capillary and then blowing them out after several hours, also seems to be carried out utilizing this hardening of cell

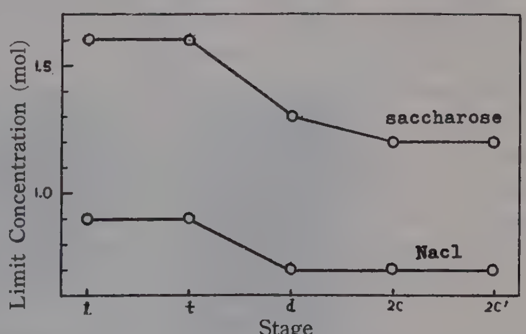


Fig. 1. Limit concentration for plasmorrhysis or plasmolysis at several developmental stages of *Coccophora* eggs. l, stage of liberation; t, stage of transformation after fertilization; d, stage of the first nuclear division but before formation of segmentation membrane; 2c, two-cell stage; 2c′, later two-cell stage.

* Biology Department, Yamagata University, Yamagata, Japan.

membrane during the time in which the eggs are contained in the capillary.

Morphogenetic movement occurs after fertilization so that the egg form becomes ovate pointed at one end (Fig. 2B). At this stage, the plasmolysis pattern usually appears at the pointed end and extends gradually (Figs. 2B′, 3A). After morphogenetic movement, nuclear division takes place and the two daughter nuclei become located along the long axis (Figs. 2C, 3B). At this stage, plasmolysis occurs not only at the pointed end, but also in the equatorial region intervening between the daughter nuclei (Figs. 2C′, 3B). This cannot be explained from the standpoint that plasmolysis is liable to occur usually at the pointed end. That is, though the egg form is not changed through mitosis, the plasmolytic pattern appears different before and after the division. Thus, it must be attributed to some physical change in the properties of the cytoplasm composing the equatorial zone. It is well-known that both spindle and cytoaster are composed of gel which is different from the surrounding cytoplasm which consists of sol[5]). Therefore, if we draw two circles of a certain radius, each originating at the center of each cytoaster, the area contained in these circles stands for the territory of the cytoasters, i.e. the zone of gel (Fig. 4). Then it is obvious that the equatorial zone is getting out of the boundary of the gel territory. In other words, that zone is considered to be of lesser viscosity. On the other hand, it is also known that the lower the viscosity of cytoplasm is the more easily plasmolysis takes place. That is to say, the plasmolysis peculiar to the equatorial zone seems to be caused by the local lowering in viscosity of cytoplasm in that region.

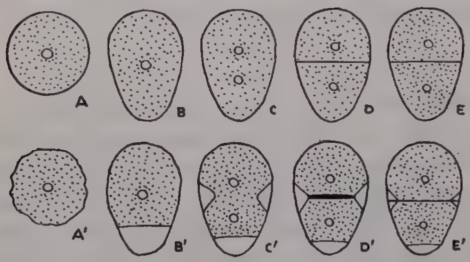


Fig. 2. A–E, normal development of the *Coccophora* egg. A′–E′, protoplasm contraction in hypertonic solution at each stage corresponding to the above.

After the nuclear division, the first segmentation occurs forming a membrane at right angles to the long axis. The new membrane is observed to be connected with the membrane of the mother cell. Nevertheless, the plasmolysis appears in just the same pattern as that which appeared before occurrence of the segmentation. That is, the egg undergoes plasmolysis at the segmentation wall as well as at the pointed

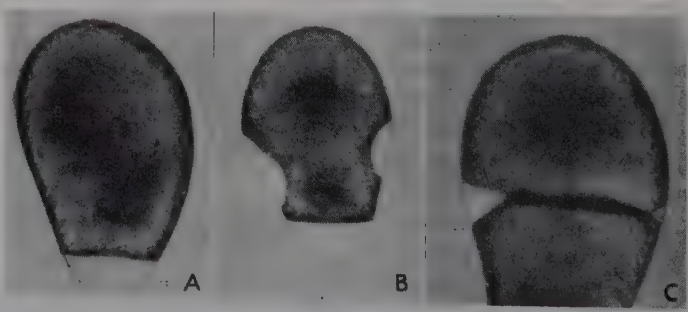


Fig. 3. A, polar plasmolysis at the pointed end; B, plasmolysis both at the pointed end and at the equatorial part; C, separation of the segmentation wall from the wall of the mother cell upon plasmolysis.

end (Figs. 2D′, 3C). On this occasion, rather curiously, the segmentation wall is separated from the wall of the mother cell at their junction with contraction of the protoplasm at the equatorial zone. Naturally, the segmentation wall also contracts to

shorter but more thickened appearances (Fig. 2D′). Later, cellulose is deposited also in the segmentation wall. Reaching this stage, it is connected firmly with the wall of the mother cell so that it cannot be separated from the latter upon occurrence of the plasmolysis (Fig. 2E′). In *Fucus* eggs, the rhizoid pole is determined on the shaded side when the egg is illuminated unilaterally with ultra-violet light. At the same time, plasmolysis appears also on the shaded side though the egg is still of a spherical form when it is immersed in a hypertonic solution[6]). It is a matter of course that this polar plasmolysis is attributed merely to a local change in the physical properties of the protoplasm. In *Coccophora*, however, the question is not always simple. That is, the polar plasmolysis, occurring at the pointed end, seems to be connected partly with the regional change in viscosity of the protoplasm but partly with the form of the egg. According to Wartenberg[7]), plasmolysis is not the separation of plasma membrane from the cell wall, but it is the development of large vacuoles between the cortex and the endoplasm. Therefore, he remarks, although it looks as if they were separated from each other, the cortex of protoplasm is still connected with the cell wall. This, however, cannot be applied to the case of fucoid eggs. As is seen in Figures 2D′ and 3C, and as was aforementioned, the segmentation wall is completely separated from the wall of the mother cell. Here, if the cortex of the protoplasm still remained attached to the cell wall, the segmentation wall would also remain together with the cortex, as it was connected with the wall of the mother cell. But the fact is different.

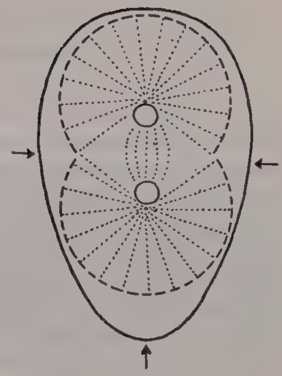


Fig. 4. Diagram indicating the territory of cytoasters after the first nuclear division. Arrows represent zones getting out of the cytoaster territory, where plasmolysis is most liable to occur.

Summary

As a result of plasmolysis experiments in eggs of *Coccophora Langsdorfii*, the following was revealed. (1) The permeability of the egg protoplasm to NaCl and to saccharose decreases after occurrence of the fertilization. (2) plasmolysis is liable to occur at the pointed end, i.e. the rhizoid pole, after the egg was transformed to an ovate form by the morphogenetic movement. (3) After occurrence of the first nuclear division, the plasmolysis occurs along the equatorial zone as well as at the rhizoid pole, though the form of the egg is identical with the former stage. This indicates that a certain change in the physical properties of the cytoplasm is taking place locally in that zone. (4) At an early stage after the first segmentation, the wall of segmentation is not firmly connected with the wall of the mother cell, so that it can be separated from the latter with contraction of the protoplasm upon plasmolysis.

References

1) Nakazawa, S., Bull. Yamagata Univ. Nat. Sci. **2**: 225 (1953). 2) Levring, T., Physiol. Plantarum **5**: 528 (1952). 3) Nakazawa, S., Bot. Mag. Tokyo **71**: 23 (1958). 4) Whitaker, D. M., Biol. Bull. **78**: 111 (1940). 5) Heilbrunn, L. V., The Dynamics of Living Protoplasm. N. Y. (1956). 6) Whitaker, D. M., Journ. Gen. Physiol. **24**: 268 (1941). 7) Wartenberg, A., Protoplasma **49**: 73 (1957).

摘　　要

フークス科藻類の発生力学 XIV. スギモク卵における原形質分離像

中　沢　信　午

スギモク (*Coccophora Langsdorfii*) の卵について原形質分離の実験をおこなつた結果，つぎのことが知られた. (1) 食塩およびしよ糖に対する卵原形質の透過性は受精後に低下する. (2) 造形運動によつて卵形に変化すると，原形質分離は卵のとがつた部分つまり仮根形成部でおこりやすくなる. (3) 第一次核分裂の直後には，卵の形態は分裂前と変りないにもかかわらず，原形質分離は仮根極のみならず赤道帯のところにもおこる. これは赤道帯のところで部分的に細胞質の物理的性質に変化がおきていることを示す. (4) 第一次卵割の直後には卵割膜は母細胞の膜とつよくは結合していない. したがつて原形質分離のときに原形質の収縮とともに母細胞の膜からはなれる. (山形大学生物学教室)

Interrelationships between Leaf Amount, Light Distribution and Total Photosynthesis in a Plant Community*

by Toshiro SAEKI**

Received August 6, 1959

Total photosynthesis and production of matter in a plant community are of primary importance for the ecological studies on the plant community. Their direct measurement must, however, be accompanied by numerous difficulties and has so far been done only in a few cultivated plants[1,2,3,4,5]), because such troublesome procedure is not practical in every research on natural vegetation. Among many environmental factors influencing the total photosynthesis of a plant community, light factor is of supreme importance in our mesophytic condition, because ' reaction ' of the plant community is, in its degree, most extensive upon the light factor as reported in a previous paper[6]). Therefore, clearing up the interrelations between leaf amount, light distribution and total photosynthesis in a plant community will provide a convenient and practical means of estimation of the total photosynthesis and, in reverse, a logical explanation to the variability of directly measured data on the total photosynthesis and dry-matter production. Such attempt has already been taken on trial by Monsi and Saeki (1953)[6]), and Davidson and Philip (1958)[7]).

In the present paper the logic will be made stricter and the mathematical equations introduced be improved to be more comparable with dry-matter increase taking place in nature than in a previous paper[6]).

Relation between leaf amount and light distribution in a plant community

Within a plant community incident light intensity diminishes on account of light absorption, for the most part, by leaf laminae. So the area of the leaves and their mode of distribution are no doubt the major determinant of light distribution inside the plant community. Now, let F be the total leaf area per unit stand area from the top to a plain x cm. above ground level. F at ground level ($x=0$) is total leaf area of the whole plants per unit stand area, that is ' leaf area index' (LAI). Then the mean relative light intensity I'/I_0 prevailing at a height of x cm. can be represented as a function of F; $I'/I_0=f(F)$. In every field work this mean relative light intensity-foliage relationship can be readily determined by means of the ' stratifying clip method '[6]) and two photometers which are employed for measuring relative light intensity. The light intensity I' thus measured at horizontal plane is, however, not always the same as the light intensity I received by the leaves at the same height, as is easily demonstrated in the case of grass communities which do not carry horizontal leaves but inclined ones. Light-photosynthesis curves so far obtained are in general plotted against illumination to which a leaf lamina had been vertically exposed to measure the photosynthesis. It is therefore necessary for the estimation of total photosynthesis of a plant community, to obtain such illumination intensity at each leaf surface in natural conditions. This can be calculated from the relative light

* Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.
** Supported by the Grant in Aid of Scientific Research of the Ministry of Education.

intensity-foliage curve through the following theoretical consideration.

Between two different heights in a stand, difference in light intensity is regarded as difference in light quantity per unit area of horizontal plane (cf. also (6)). If the two heights are replaced by F and $F+\Delta F$, this difference consists of fractions absorbed and reflected back to sky by ΔF. So, the mean light quantity per unit leaf area which the leaves between the two heights should absorb and reflect is $[f(F)-f(F+\Delta F)]I_0/\Delta F$. At infinitesimal of ΔF, the light quantity can be expressed as $-I_0 f'(F)$, i.e., the light intensity equivalent to the absorbed and reflected fractions. The light intensity to be received by the leaves at this height is the sum of the two fractions and the transmitted fraction of light. So, when m stands for leaf transmissibility, the received relative light intensity I/I_0 is expressed as

$$I/I_0=-f'(F)/(1-m)\,. \qquad (1)$$

This basic formula of light penetration implicates the fact that under constant incident light intensity the more the light is absorbed and reflected by each leaf, the less the light penetrates below the foliage, and *vice versa*. This formula is applicable to every case, whatever the form of function $f(F)$ may be, and even to heterogeneous plant communities. In the case of a plant community carrying leaves of horizontal habit, I/I_0 may not be so different from I'/I_0 and the former can be justifiably replaced with the latter, while in a grass-type community this replacement fails. For this reason, it seems to be disputable that as the light received by leaves Takeda adopted I'/I_0 to a grass-type community such as rice plants[7]. On the other hand, for simplifying the calculation of illumination in buckwheat stands, Iwaki assumed that the leaves from $F=0$ to $F=1$ received full daylight[9]. If so, at $F=1$ residual light quantity must originate exclusively from transmitted fraction, and the illumination which the leaves in the range $1<F<2$ received would have to be less than the leaf transmissibility of this plant, i.e. 8%, yet he assumed the value to be 40.5%. Such erroneous procedures may lead to mistakes in conclusion. In phytoplankton the measurements of photosynthesis rate and light intensity are always made in community state, so that the distinction between I'/I_0 and I/I_0 is unnecessary and the extended application of Equation (1) to phytoplankton community as attempted in a previous paper[10]) is misleading. Fig. 1 where the productive structure of *Miscanthus sacchariflorus* community, a typical lowland tall-grass community in central Japan, is illustrated, gives a clear comparison between the distribution curve of I/I_0 and that of I'/I_0 in the plant community.

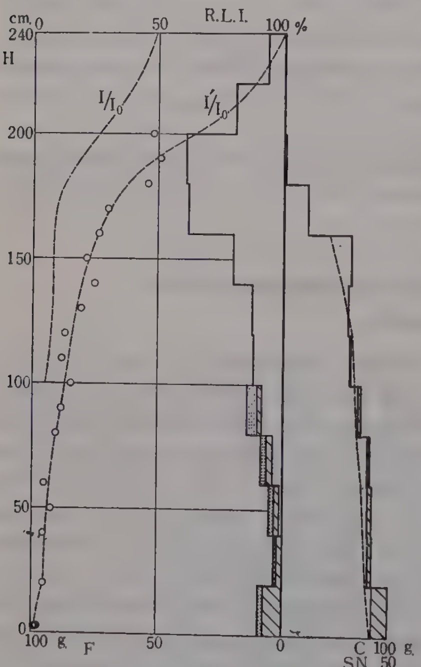


Fig. 1. Productive structure of a *Miscanthus sacchariflorus* community. Two broken lines show the contrast between the distribution of relative light intensity measured at horizontal plane (I'/I_0) and the distribution derived from Equation (1) of the relative light intensity to be received by leaves (I/I_0). Leaf transmissibility (m) in this plant is 10%[11]). The hatched area, the system of other species; the dotted, the yellowing leaves. SN means stem number of *Miscanthus* plants.

After surveys extending a wide range of herb communities in Japan, Monsi and Saeki[6]) have revealed that in a homogeneous community the function $f(F)$ can be expressed as follows:

$$f(F)=I'/I_0=\exp(-KF)\,. \qquad (2)$$

This equation means $\ln I'/I_0$ linearly relates to F. Further theoretical analysis, however, indicated that in a plant community having steeply inclined leaves, theoretical relation between $\ln I'/I_0$ and F diverges from perfect linearity: In a range of higher F, I'/I_0 is theoretically somewhat higher than expected from Equation (2). In natural stands, however, when upper younger leaves are erect, lower older ones are more and more inclined towards horizontal, thereby contributing to establishment of Equation (2). Moreover, theoretical analysis indicated that the extinction coefficient K is determined by transmissibility, arrangement and especially inclination of leaves. When Equation (2) is substituted into Equation (1), illumination intensity at the surface of leaf lamina is expressed in terms of F, m and K as

$$I/I_0=K\exp(-KF)/(1-m)\,. \qquad (3)$$

If K and m are known, vertical distribution of I/I_0 will be easily derived from this equation by the use of parameter F. Davidson and Philip[7]) adopted Equation (2) but not Equation (3) to the theoretical analysis of pasture growth, so their results can hold only for stands of plants with broad leaves of approximately horizontal habit, such as *Trifolium subterraneum* discussed in their study.

Total photosynthesis and leaf amount in a plant community

In a previous paper[6]) it was assumed that all the leaves within a plant community give the same light-photosynthesis curve, but it has been clarified by further investigations that this assumption is inadequate, because the duration of high photosynthetic activity is very short in annual plants and, as well known, differentiation of sun and shade leaves is often remarkable in trees. However, so far as plants construct a dense plant community this assumption does not lead to any essential error, in other words, light-photosynthesis relation in every leaf can be represented practically with one and the same gross photosynthesis curve which is obtained with an active leaf, and the respiration, with the mean respiration rate of all leaves as already reported[12]).

This conclusion makes it easy to integrate photosynthesis of each leaf and to obtain the total photosynthesis of the foliage of a plant community. By combining an hourly photosynthesis curve in an active leaf (Fig. 2) with mean daily march of illumination (Fig. 3), a relative light intensity-daily photosynthesis curve can be constructed. Two such examples calculated from the data on *Celosia cristata* and *Zelkowa serrata* are both illustrated in Fig. 4, where daily photosynthesis is represented by gain in $(C_6H_{10}O_5)_n$ so as to be comparable with plant growth in dry weight. The hourly photosynthesis curves for the two species illustrated in Fig. 2 can be regarded as representatives of herbaceous plants and broad-leaved trees, respectively, because the light-photosynthesis curves obtained in our laboratory for about 80 species revealed that the mean value of photosynthetic capacity (light-saturated net photosynthesis at normal atmospheric CO_2 concentration) in herbaceous plants is 7–8 mg. and that in trees is 5–6 mg. CO_2/50 sq. cm./hr., at 25° (unpublished). As for mean daily march of incident light intensity, an average of mean daily course of illumination measured in Tokyo by Hirayama during four months from June to September in 1940–1941[13]) was used (Fig. 3).

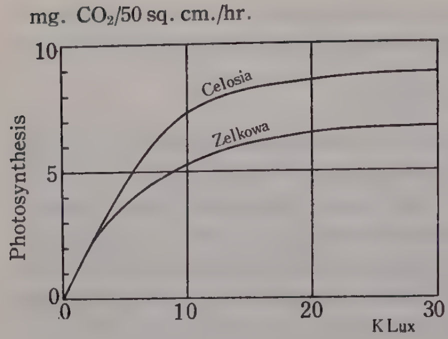


Fig. 2. Light-real photosynthesis curves in the active leaf of *Celosia cristata* and *Zelkowa serrata* at 25° and 0.03% CO_2. Mean respiration rate is 0.65 mg. CO_2/50 sq.cm./hr. in *Celosia* (in consideration of leaf weight[12]) and 0.50 mg. in *Zelkowa* (mean value for sun and shade leaf).

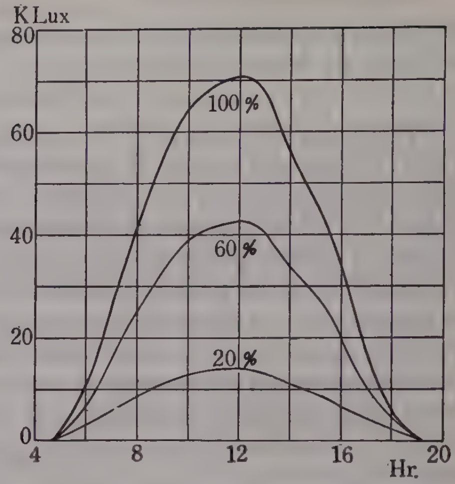


Fig. 3. Mean diurnal courses of light intensity in 100%, 60% and 20% of incident light. The curve for 100% corresponds to mean diurnal course of light intensity for four months in summer measured at Tokyo[13]).

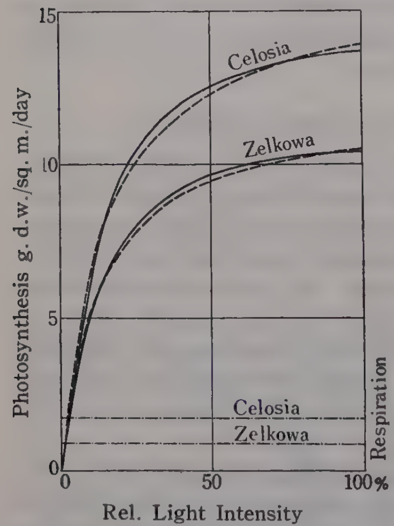


Fig. 4. Relative light intensity-daily real photosynthesis curves in *Celosia* and *Zelkowa*. Solid lines; the values calculated from Figs. 2 and 3. Broken lines; the curves of rectangular hyperbola with which the solid lines were approximated. Chain lines; the level of mean daily respiration at 25°. If 100% light intensity is represented by $I=1$, the constants in Equation (4) are as follows: *Celosia*; $a=6.9$, $b=110$ and $\bar{r}=1.92$. *Zelkowa*; $a=7.7$, $b=91.2$ and $\bar{r}=1.47$.

As drawn with broken lines in Fig. 4, the light-photosynthesis curves thus obtained can well be approximated with rectangular hyperbolae (cf. Davidson and Philip[7]),

$$q=\frac{bI}{1+aI}-\bar{r}, \qquad (4)$$

where a and b are constants which characterize the shape of the curve, and $\bar{r}$ is the mean respiration rate of all the leaves. Such rectangular hyperbolic curve was already applied to hourly light-photosynthesis curve in a previous paper[6]).

Insertion of Equation (3) into Equation (4) and its integration with respect to F provide the mean total daily photosynthesis P of all the leaves as a whole (daily surplus production) in a plant community whose LAI is F, that is,

$$P=\frac{b}{Ka}\ln\frac{(1-m)+KaI_0}{(1-m)+KaI_0\exp(-KF)}-\bar{r}F. \qquad (5)$$

In the right hand side of this equation, the first term expresses daily gross production, i.e., total sum of real assimilation of all the laves. Fig. 5 shows P as function of F and K at 100% incident light, other quantities assuming the values for *Celosia* used in Fig. 4. As shown in the curves in Fig. 5, P increases with increase of F to some extent. The relation is not linear but convex, which means increasing mutual shading of foliage leaves with increase of F. Each of these curves has a maximum, beyond

which the surplus production decreases on account of negative assimilation in the deeply shaded lower leaves. Furthermore, these curves evidently indicate that in lower F daily production in foliage is not much different among plant species having different K, but with increase of F these differences become very remarkable. This result clearly interpretes the difference in growth between sugar beet and the wild types of sea beet reported by Watson and Witts[14]), because the former has upright leaves (smaller K[6])) while the latter have prostrate ones (larger K). According to their experiment, in a stage of lower F, NAR was almost the same in both species, but in a later stage of higher F, NAR in sugar beet was much higher than that in the wild types. Equation (5) also indicates that the daily surplus production increases with increasing radiation level, in a convex but less curved curve than in datached leaf. Similar relation for momentary photosynthesis has already been demonstrated experimentally by Boysen Jensen[1]), Takeda and Maruta[3]) and Yamada *et al.*[5]). In a similar equation in a previous study[6]), P was expressed as hourly photosynthesis of whole foliage in an illumination of a definite intensity, while in the present paper P means the daily surplus production in a plant community and I_0, the incoming radiation level relative to the mean daily illumination in summer period of Tokyo (the 100% curve in Fig. 3, 568,000 lux-hours).

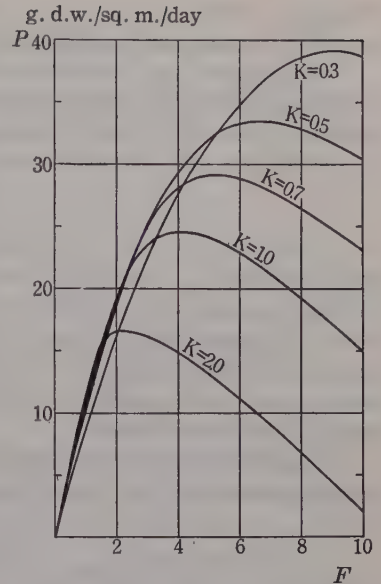


Fig. 5. Daily surplus production P of *Celosia* stand under full daylight (100%) calculated from Equation (5). F in abscissa is 'leaf area index'. K is extinction coefficient. Leaf transmissibility $m=0.1$, other constants being the same as in Fig. 4.

Light-compensation point, optimum leaf amount and maximum production in a plant community

The maximal daily surplus production is performed by a leaf amount where the lowermost leaves assimilate dry matter in average just to compensate for daily matter loss by respiration ($q=0$). The leaf amount has been denoted as F_{opt}, when expressed in LAI[6]). When LAI exceeds F_{opt}, the lower leaves exceeding the F_{opt} probably perish and fall in due time on account of dry-matter loss by respiration surpassing photosynthetic gain. In this respect the information presented by Verikof[15]) should be noticed. Studying translocation and distribution of assimilated substances in soybean plants, he clarified the substances assimilated by a light exposed leaf were hardly furnished to the neighboring starved leaves. Provided this result is applicable to other plants the assumption introduced by Davidson and Philip will be denied that the leaves situated lower than compensation depth can survive until total respiration of a whole stand exceeds its total gross photosynthesis[7]). Therefore, F_{opt} is not only the 'optimum LAI' for dry-matter production in the plant community but also the maximum LAI to be able soundly to exist. Davidson and Philip reported that irrigated swards at Adelaide developed foliage with a maximal LAI of about 10[7]). Takeda and Kumura also demonstrated that rice plants at heavy manuring could bear leaves cor-

responding about 10 in LAI[8]). Such exceedingly high LAI will rarely appear and rather as a transient phenomenon in most cases.

The relative light intensity at this compensation point, I_c, can be obtained by combining daily gross photosynthesis curve (this can be represented by the curve for an active leaf e.g. in Fig. 4 because of weak illumination) and daily respiration r of the lowest but not yellowish leaf. Then,

$$\frac{bI_c}{1+aI_c}=r\,. \tag{6}$$

The values of I_c calculated from Equation (6) were 1.7% and 1.1% in *Celosia* and *Zelkowa*, respectively. As above mentioned, the data on these two species can be taken as representative ones of vigorously growing plants in our temperate zone. The relative illuminations on the stand floor of a large number of our herbaceous communities were generally 2–3%[6]). The calculated compensation points, therefore, seem to be a little smaller.

A rough estimate of the value of 'optimum LAI' F_{opt} (F at $q=0$) can be obtained from Equations (3) and (6) as follows:

$$F_{opt}=\frac{1}{K}\ln\frac{KI_0(b-ar)}{r(1-m)}\,. \tag{7}$$

The values of the F_{opt} (Fig. 6) calculated by means of Equation (7) from the data for *Celosia* and *Zelkowa* were 4–11 (in the range from $K=1$ to $K=0.3$) and a little larger than the LAI 4–7 which were found in natural herbaceous communities in Japan[6]). The smaller values of compensation light intensity and the larger values of 'optimum LAI' calculated are possibly due to the following reasons: Light intensity distribution at the same stratum in a stand shows in practice deviations from the mathematical mean, which are noticeable in presence of sunflecks in the lower layer. Light-photosynthesis curves are always convex upwards, so if mathematical mean of light intensities is taken, the calculated photosynthesis of a plant community as a whole should be somewhat overestimated. Other reasons to be considered may be elevation of compensation light intensity of the lower leaves due to (1) additional respiratory loss of dry matter in non-photosynthetic systems such as stem and root, (2) continued cloudy or rainy weather which may occur occasionally during growing period and (3) shading by plant organs other than leaf laminae.

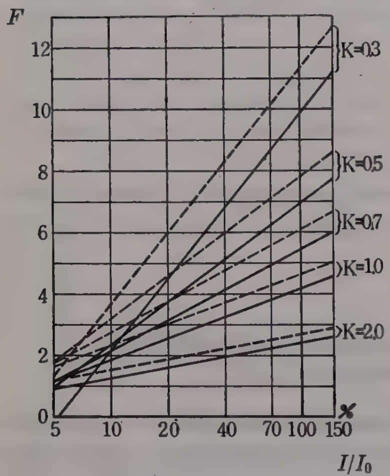


Fig. 6. 'Optimum leaf area index' F plotted against relative light intensity I/I_0 in logarithmic scale. Calculated by means of Equation (7) and constants derived from *Celosia* (solid lines) and *Zelkowa* (broken lines). r and m are 1.71 (g/sq. m./day)[12]) and 0.1 in *Celosia*, and 0.88 and 0.07[11]) in *Zelkowa*, respectively, other constants being the same as in Fig. 4.

The daily maximum surplus production (denoted with P_{max}) is performed by 'optimum LAI' as mentioned above. The small overestimation of F_{opt} exerts little effect on P_{max}, because contribution of the lower leaves to productivity of the whole stand is very small. Then, Equations (5) and (7) provide P_{max} as follows:

$$P_{max} \doteqdot \frac{b}{Ka} \ln \frac{1+aKI_0/(1-m)}{1+ar/(b-ar)} - \frac{\bar{r}}{K} \ln \frac{KI_0(b-ar)}{r(1-m)} \, . \tag{8}$$

From Figs. 7 and 8 in which the dependence of P_{max} on I_0 and that on K are plotted,

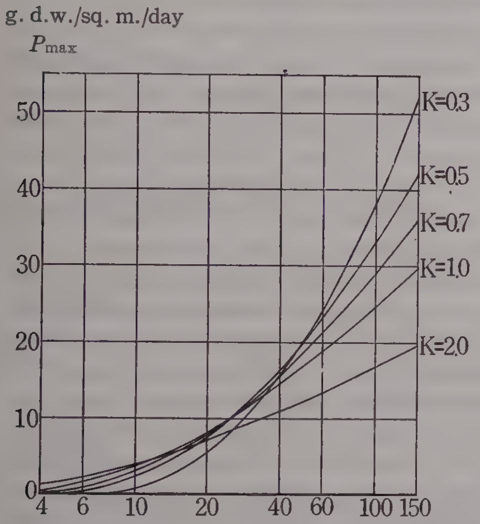


Fig. 7. Daily maximum surplus production P_{max} in relation to relative level of incident radiation (I_0) at different extinction coefficient K. Calculated from equation (8). The constants are the same as in *Celosia* in Fig. 4.

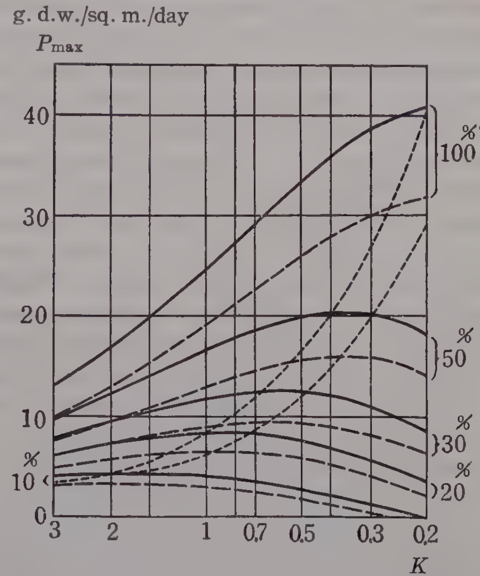


Fig. 8. Daily maximum surplus production P_{max} in *Celosia* stand (solid lines) and *Zelkowa* stand (broken lines) in relation to extinction coefficient K at relative light intensities of 100%, 50%, 30%, 20% and 10%. Calculated from Equation (8).

it is evident that P_{max} is markedly different among plant communities having different extinction coefficients or exposed by different light intensities. In each fixed course of daily incoming radiation, P_{max} has again one maximum at $dP_{max}/dK=0$, where $KI_0/(1-m)$ is constant (Fig. 8). At high light intensities, therefore, P_{max} is higher in a plant community having a smaller K, on the other hand at low light intensities the maximum of P_{max} is found in a plant community having a larger K, as mentioned in a previous paper[6]). For dry-matter production in natural vegetation, therefore, it is of greater significance that in uncovered situations the plants, as is often the case, have inclined leaves, on the contrary the leaves in the deep shade are horizontally arranged.

The calculated surplus production (Figs. 6, 7, 8) will be applicable to any plant community in a given radiation level, if photosynthetic capacity in an active mature leaf is in the same level as in *Celosia* or *Zelkowa* (see Fig. 2) and water condition is favourable. Midorikawa[16]) has studied productivity of an *Aconitum* altherbosum on Mt. Hakkoda (northern Honshu) and reported that, when leaf amount attained to the highest level in late June, the daily gross production and surplus production were 35 and 28 g. d.w./sq. m./day, respectively. In this plant community I_0 was reported about 100% and K, 0.6–0.8. Regrettably photosynthetic capacity of *Aconitum* leaf has not been reported, but the value can be estimated from Figs. 8 and 2 at 8 mg. CO_2/50 sq. cm./hr. in net rate, a little smaller value than 8.3 mg. in *Celosia*. The magnitude of this estimated value is not beyond expectation as stated above.

Blackman and Black[17]) collated the maximal levels of daily net production measured in U.S.A. and England by different workers. Those values may serve as standards of comparison with the theoretical values of P_{max} presented here, because they can be regarded as achieved by 'optimum LAI' under good water and nutrient conditions. Although basic data such as photosynthetic capacity of leaf and extinction coefficient in the plants concerned were not provided, Verduin and Loomis in other experiment obtained in average 7.9 mg. CO_2/50 sq. cm./hr. as photosynthetic capacity of corn[18]). Photosynthetic capacity in cotton and sugar beet attained to 10 mg. per the same unit[19]). Therefore, it may be of interest to compare the cited data with values calculated in *Celosia* (photosynthetic capacity 8.3 mg.) at a radiation level of 100%. Of the plants cited, sugar cane, corn, sugar beet and barley have steeply inclined leaves, while cotton and kale have relatively less inclined leaves. In herbaceous communities in Japan K is usually 0.3–0.5 in stands with steeply inclined leaves and 0.7–1.0 in stands with less inclined leaves[6]). Therefore, quite reasonable is the assumption that the extinction coefficient in the former species is 0.4 and that in the latter species is 0.8. From Fig. 8 the daily surplus production in *Celosia* is 35 g./sq. m. at $K=0.4$ and 27 g. at $K=0.8$. If total respiration in non-phtosynthetic system (stem, petiole, root etc.) is assumed 1/6 of the surplus production, then the net production at $K=0.4$ and $K=0.8$ will be 29 g. and 23 g./sq. m., respectively. In such high radiation level as 100%, gross production far exceeds total respiration, so, as seen from Equation (8), P_{max} is nearly proportional to the value b which is also roughly proportional to photosynthetic capacity. Therefore, when photosynthetic capacity is raised from 8.3 mg. CO_2/50 sq. cm./hr. to 10 mg., net production is calculated at $K=0.4$ to be $35\times 10/8.3\times 5/6=$ 35 g./sq. m. and at $K=0.8$ to be 27 g. These theoretical values coincide considerably well with those measured in field experiment (sugar cane 38 g./sq. m., corn 27 g., sugar beet 31 g., barley 23 g., cotton 27 g., kale 21 g.). The larger value, 38 g./sq. m., measured in sugar cane[20]) may be ascribed to the higher mean radiation in Hawaii as already conjectured by Blackman and Black[17]) (cf. also Fig. 7).

Summary

1. In order to find a practical means of estimation of the production of matter in a plant community and to give a logical explanation to the variability of directly measured values, theoretical analyses have been advanced of the interrelationships between leaf amount, light distribution and total foliage photosynthesis.

2. Inside foliage relative light intensity received by the leaves is not always the same as the light intensity measured at horizontal plane at the same height (Fig. 1). In homogeneous stands the former can be derived from Equation (3), when leaf transmissibility is known and extinction coefficient (K in Equation (2)) is obtained beforehand by 'stratifying clip method'.

3. If photosynthetic capacity in the active leaf and mean respiration rate of all the leaves in a stand are known, the mean total daily photosynthesis of whole foliage is estimated by Equation (5). An example in representative herbaceous species is presented in Fig. 5, where it is clearly indicated that with lower 'leaf area index' daily production in foliage is indifferent to inclination of leaves, while with increase of leaf amount the role of inclination in the production becomes very remarkable, upright leaves being more efficient than horizontal ones under full daylight as demonstrated by Watson and Witts.

4. Compensation light intensity and 'optimum leaf-area index' (F_{opt}—leaf amount

in the form of LAI for the highest production) are calculated from the photosynthetic capacity in the active leaf and respiration rate of the lower leaf (Equations (6) and (7)). The obtained values seem to be quite reasonable in consideration of the minimum light intensities and 'leaf area indexes' in the natural communities.

5. The highest daily production in a plant community, P_{max}, calculated with Equation (8) was discussed in relation to the extinction coefficient and incoming radiation (Figs. 7 and 8). An approximate coincidence was recognized between the calculated values and the highest net production in crop fields collated by Blackman and Black.

The author should like to express his sincere thanks to Prof. M. Monsi for his invaluable advice and encouragements.

References

1) Boysen Jensen, P., Stoffproduktion der Pflanzen, Jena (1932). 2) Thomas, M. D. and Hill, G. R., Photosynthesis in Plants, Iowa: 19 (1949). 3) Takeda, T. and Maruta, H., Proc. Crop Sci. Soc. Jap. **24**: 34 (1955). 4) ——, ibid. **24**: 331 (1956). 5) Yamada, N., Murata, Y., Osada, B. and Iyama, J., Proc. Crop Sci. Soc. Jap. **24**: 246 (1956). 6) Monsi, M. and Saeki, T., Jap. Journ. Bot. **14**: 22 (1953). 7) Davidson, J. L. and Philip, J. R., Climatology and Microclimatology, UNESCO: 181 (1958). 8) Takeda, T. and Kumura, A., Proc. crop Sci. Soc. Jap. **26**: 165 (1957). 9) Iwaki, H., Jap. Journ. Bot. **16**: 210 (1958). 10) Saeki, T. and Kuroiwa, S., Bot. Mag. Tokyo **72**: 27 (1959). 11) Kasanaga, H. and Monsi, M., Jap. Journ. Bot. **14**: 304 (1954). 12) Saeki, T., Bot. Mag. Tokyo **72**: 404 (1959). 13) Hirayama, T., Theoretical Architecture (Japanese: Kenchiku-Sekkei Riron), Tokyo (1948). 14) Watson, D. J. and Witts, K. J., Ann. Bot. 23: 431 (1959). 15) Verikof, E. F., Plant Physiology, Moscow **2**: 354 (1955). 16) Midorikawa, B., Ecol. Rev. **15**: 83 (1959). 17) Blackman, G. E. and Black, J. N., Ann. Bot. **23**: 131 (1959). 18) Verduin, J. and Loomis, W. E., Plant Physiol. **19**: 278 (1944). 19) Böhning, R. H. and Burnside, C. A., Am. Journ. Bot. **43**: 557 (1956). 20) Borden, R. J., The Plant Crop. Haw. Plant Rec., **46**: 191 (1942).

摘　　要

植物群落における葉量，光分布，全光合成の相互関係

佐　伯　敏　郎

植物群落における物質生産を葉量，光分布，葉の光合成能力等の基本的要因から導く方法を理論的に明らかにするのが本論文の目的であるが，これは同時に植物群落の物質生産の種々の実測値に対して正しい説明を与える．

本論文ではまず植物群落内で測定される照度分布は必ずしも葉が実際に受ける照度の分布とは一致しないことを強調し，後者は前者の値とそれを規定する葉量との関係から導きうることを示した．[式 (1)].

壮葉での光合成能力と全葉の平均呼吸率が与えられれば，日射の日変化をこれに結びつけることによつて，全光合成を，地上単位面積当りの乾量増加の形で与える式 (5) が理論的にえられる．この式によれば植物群落の全光合成は上記日射の強さと葉の光合成能力の他に葉量と吸光係数 [式 (2) の K] により大きく影響されることが分る．また光補償点まで葉が存在するとき，全光合成は最大になり，このときの光補償点，葉量 (F_{opt})，全光合成 (P_{max}) が同様に容易に導かれる [式 (6), (7), (8)].

以上の式に草本の代表としてケイトウの，木本の代表としてケヤキの光合成能力および呼吸率を適用して数値計算をしたところ (図 5, 6, 7, 8)．100% の光条件のもとで，光補償点は 1～2%，最適葉面積指数は 4～11 (K=0.3～1.0 として) またこの時の純生産として 23～35 g./sq. m./day (K=0.4～0.8, 単葉の見かけの光合成を 8.6～10 mg. CO_2/50 sq. cm./hr. として) がえられ，各地で測定された好条件下の植物群落での実測値にかなりよくあてはまることが見出だされた．（東京大学理学部植物学教室）

Comparative Effectiveness of Gibberellins A_1, A_2, A_3 and A_4, with Special Reference to That of A_4*

by Tohru HASHIMOTO** and Toshio YAMAKI**

Received August 17, 1959

The gibberellins, which are plant growth stimulators, were isolated as a crude crystalline mixture from culture filtrates of the pathological fungus *Gibberella fujikuroi* Wr. in 1935. Since that time four gibberellins, i.e., A_1, A_2, A_3 and A_4 (GA_1, GA_2, GA_3 and GA_4) were isolated.

Meanwhile it has been revealed that the gibberellins which were thus isolated as extensive growth stimulators of rice seedlings have many physiological effects such as stimulation of stem elongation and leaf expansion, growth promotion of dwarf mutants, removal of light inhibition in *Avena* first internodes, promotion of flowering, fruit setting, induction of dark germination of light sensitive seeds, etc. The historical background of discoveries and the physiological activities of the gibberellins have been reviewed by Stowe and Yamaki[1,2]) and Brian[3]).

The investigations of these physiological effects were performed by using GA_3 (gibberellic acid) or a mixture of GA_1 and GA_3. The next step would be to determine whether or not all of the four gibberellins have these physiological activities, and if active, how the comparative effectiveness is.

Bukovac and Wittwer[4]) investigated the effects of GA_1, GA_2, GA_3, GA_4 and methylesters of GA_1 and GA_3 in inducing the vegetative elongation of epicotyls, flowering of facultative long-day annuals and fruit setting. According to their experiment, all of these gibberellins and the methylesters were effective in the investigated phenomena, and the order of activity was $GA_3 > GA_1 \geqq GA_4 > GA_2 >$ methylester $A_1 \geqq$ methylester A_3. Sumiki[5]) also reported that the effectiveness was in the order of GA_3, GA_1, GA_4 and GA_2 in promoting the elongation of rice seedlings. Phinney and Neely[6]) obtained a similar order of effectiveness by the application of GA_1, GA_2 and GA_3 to the seedlings of dwarf mutant and normal maize.

We previously reported[7]) in brief that all of the four gibberellins were effective in promoting leaf expansion and inducing the dark germination of light sensitive tobacco seeds, and that GA_4 was the most active in comparison with the other gibberellins. The present paper describes the detailed data about that experiment, and also gives the results obtained thereafter.

Materials and Methods

Comparative effectiveness of these gibberellins was studied with the elongation of intact rice seedlings, the expansion of radish leaf disks and etiolated bean leaf disks, and the induction of the germination of light sensitive tobacco seeds in the dark. The effects of GA_3 and of a mixture of GA_1 and GA_3 were already reported all these cases[6,8,9]) except that of bean leaf disks.

The rice employed was *Oryza sativa* L. "Norin No. 25". The seeds were sown

* The present studies were supported in part by grants from the Ministry of Education.

** Biological Institute, College of General Education, University of Tokyo, Komaba, Meguro, Tokyo, Japan.

in shallow tap water in a large Petri dish and kept at 25–30° for three days until the coleoptiles appeared and became 2–3 mm. in length. The seedlings of uniform size were selected and lots of 12 seedlings each were transferred to small Petri dishes, 5.5 cm. in diameter, containing three layers of filter paper and 10 ml. of Boysen Jensen's culture solution with or without the addition of one of the gibberellins. Two dishes were prepared for each experimental condition. All these dishes were kept in an experimental room under diffuse light at 25–30°, being occasionally supplemented with distilled water. When the seedlings grew to reach the lids of the dishes, the lids were removed. Six days after the transference of the seedlings the length of the second leaf sheath was determined.

The radish used was *Raphanus sativus* L., "Riso-daikon". The experiment was made according to Kuraishi's method[10]).

In the case of bean leaf, the experimental method was almost similar to Miller's one[11]); but 2 per cent sucrose was added to the incubation medium instead of glucose. Sucrose brings about better growth of bean leaf disks than glucose, and unlike glucose no lowering of pH of the medium during incubation was observed[12]).

Seeds of tobacco (*Nicotiana tabacum* L., "Bright Yellow") were obtained at Tateyama, Chiba Prefecture. These seeds ordinarily require exposure to light for germination. Seeds were sown on two layers of filter paper soaked with 0.02 M potassium nitrate solution containing various concentrations of gibberellins and were allowed to germinate for five days at 25° in the dark. Potassium nitrate intensifies the gibberellin-induced germination[13]).

Tested concentrations of GA_1, GA_2 and GA_3 ranged from 10^{-12} to 10^{-4} M, and those of GA_4 from 10^{-12} to 3×10^{-6} M because of its insolubility in concentrations above 3×10^{-6} M.

Results

The elongation responses of the second leaf sheath of intact rice seedlings to GA_1, GA_2, GA_3 and GA_4 are indicated in Fig. 1. Growth promotion was obtained over the concentration ranges above 10^{-7} M for GA_1 and GA_3, above 6×10^{-7} M for GA_4, and above 10^{-6} M for GA_2. As seen in the figure, GA_1 and GA_3 were far more effective than GA_2 and GA_4; GA_4 has a little stronger effect than GA_2, and no significant difference in activity was noticed between GA_1 and GA_3. In this case the order of effectiveness was $GA_1 \doteqdot GA_3 \geqslant GA_4 > GA_2$.

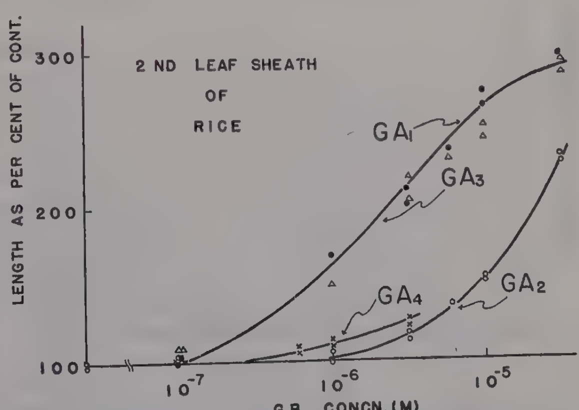


Fig. 1. Effects of GA_1, GA_2, GA_3 and GA_4 on the elongation of the second leaf sheath of rice seedling cultured for 6 days at 25–30°. Ordinate shows the length of the second leaf sheath as per cent of that of control (36.0 mm.).

In the case of application to leaf disks, on the other hand, the effectiveness order of the gibberellins was different from that for the leaf sheath elongation. Fig. 2 shows the results of the experiment about the effects of the

gibberellins on the expansion of radish leaf disks in the light. Among the four gibberellins GA_4 produced the largest acceleration of leaf expansion and its activity was noticed even at as low a concentration as 10^{-11} M. GA_3 was more effective than GA_1 in high concentrations, but at lower concentrations the reverse was the case. GA_2 was the least effective among the four gibberellins.

Growth responses of etiolated bean leaf disks to the gibberellins were similar to those of radish leaf disks. The growth promotion of bean leaf disks was observed over the similar concentration ranges, and the order of effectiveness of the four gibberellins was identical.

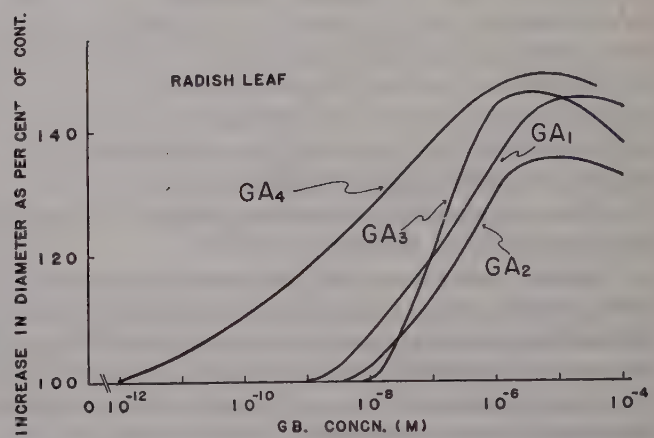


Fig. 2. Effects of GA_1, GA_2, GA_3 and GA_4 on the expansion of radish leaf disks. Ordinate shows the increase in diameter of treated disk as per cent of that of control disk (0.672 mm.) for 18 hours in the light. Initial average diameter of a disk was 4.33 mm.

Fig. 3 shows the germination rates of tobacco seeds induced by the four gibberellins in the dark. The germination was observed over the concentration ranges above 10^{-7} M, 3×10^{-6} M, and 3×10^{-5} M for GA_4, GA_1 and GA_3, and GA_2, respectively. The concentrations required for 20 per cent germination were ca. 2×10^{-6} M, 9×10^{-5} M, 10^{-4} M and 3×10^{-4} M for GA_4, GA_1, GA_3 and GA_2, respectively. It was convincingly demonstrated that GA_4 was the most active and GA_2 was the least active of the four in inducing the germination, too. The activities of GA_1 and GA_3 ranked between those of GA_4 and GA_2. Though the difference in the activities of GA_1 and GA_3 was small, as seen in Fig. 3, GA_1 produced a little higher percentage of germination in all the repeated experiments. In the experiment in which Boysen Jensen's culture solution was used as the basal medium instead of 0.02 M potassium nitrate, corresponding results were obtained.

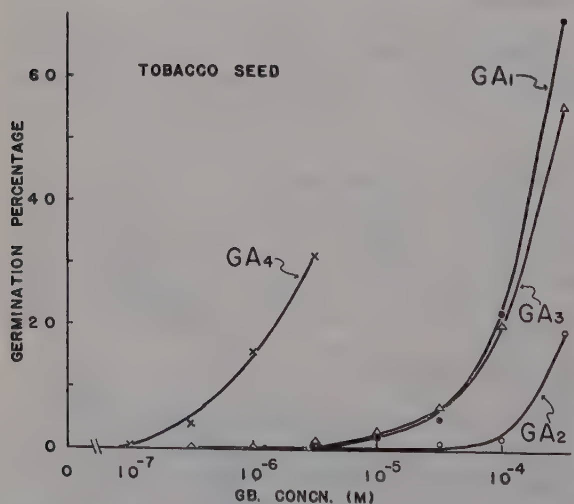


Fig. 3. Effects of GA_1, GA_2, GA_3 and GA_4 on the germination of tobacco seeds in the dark. Germination rates were determined 5 days after sowing. The medium contains 0.02 M potassium nitrate.

Discussion and Conclusion

As seen above, different concentration ranges of each gibberellin were required for the elongation of the second

leaf sheaths of rice seedlings, the expansion of leaf disks of radish and bean, and the germination of tobacco seeds, respectively. The leaf disks responded to lower concentrations of gibberellins, while the tobacco seeds required larger doses of gibberellins than the rice seedlings.

When attention is paid solely to the order of effectiveness of these four gibberellins, we find out that the order differs with the phenomena investigated here. $GA_1 \fallingdotseq GA_3 \geqslant GA_4 > GA_2$ for the growth promotion of the second leaf sheaths of rice seedlings, $GA_4 \geqslant GA_3 > GA_1 > GA_2$ for the accerelation of the leaf expansion of radish and bean, and $GA_4 \geqslant GA_1 > GA_3 > GA_2$ for the induction of the dark germination of tobacco seeds were observed. The order of the effectiveness in the rice seedlings generally corresponded to the results obtained by Bukovac and Wittwer[4]), Phinney and Neely[6]), and Sumiki[5]), except that no difference in activity between GA_1 and GA_3 was found. On the other hand, in the case of the leaf expansion and of the dark germination of tobacco seeds, the order of the effectiveness was quite different, especially in the fact that GA_4 was the most active. This is to be noted, since GA_4 had relatively little activity in promoting the elongation of rice seedlings[5]), the epicotyl elongation of bean, the stem elongation and the flowering of lettuce and dill[4]).

Though what causes such different effectiveness of GA_4 can not be mentioned from the present data, the particular characters of GA_4 for permeability and affinity to the probably existing active site in the cell and the special action mechanism for different physiological phenomena may be conceivable as the cause.

Acknowledgements

We wish to thank Prof. Y. Sumiki, University of Tokyo, for supplies of gibberellins A_1, A_2, A_3 and A_4, Prof. M. Nagao, Tohoku University, for a supply of rice seeds, and Japan Monopoly Corporation for a supply of tobacco seeds. Thanks are also due to Dr. A. Kawarada for his technical advice and suggestion.

Summary

Comparative effectiveness of gibberellins A_1, A_2, A_3 and A_4 (GA_1, GA_2, GA_3 and GA_4) in promoting the elongation of rice seedlings, in accelerating the expansion of green radish leaf disks and of etiolated bean leaf disks, and in inducing the dark germination of tobacco seeds, was investigated. These four gibberellins were all active in every investigated phenomenon, but different in comparative activity. In the leaf expansion and the tobacco seed germination, unlike in the elongation of rice seedlings, GA_4 was especially active and new orders of effectiveness such as $GA_4 \geqslant GA_3 > GA_1 > GA_2$ or $GA_4 \geqslant GA_1 > GA_3 > GA_2$ were observed. It appears that gibberellins show different effectiveness with different physiological phenomena.

References

1) Stowe, B. B. and Yamaki, T., Ann. Rev. Plant Physiol. **8**: 181 (1957). 2) Stowe, B. B. and Yamaki, T., Science **129**: 807 (1959). 3) Brian, P. W., Biol. Rev. **34**: 37 (1959). 4) Bukovac, M. J. and Wittwer, S. H., Nature **181**: 1484 (1958). 5) Sumiki, Y. Speech at the Meeting of Japan Chemical Society (1959). 6) Phinney, B. O. and Neely, P. M., Plant Physiol. 33 Suppl. XXXVIII (1958). 7) Hashimoto, T. and Yamaki, T., Bot. Mag. Tokyo **72**: 178 (1959). 8) Kuraishi, S. and Hashimoto, T., ibid **70**: 86 (1957). 9) Ogawara, K. and Ono, K., Japanese Gibberellin Research As-

sociation, First Symposium (1957). 10) Kuraishi, S. and Okumura, F. S., Bot. Mag. Tokyo **69**: 300 (1956). 11) Miller, C. O., Arch. Biochem. Biophys. **32**: 216 (1951). 12) Unpublished data. 13) Hashimoto, T., Bot. Mag. Tokyo **71**: 432 (1958).

摘　　要

ジベレリン A_1, A_2, A_3, A_4 の生理作用の比較，とくに A_4 の作用について

橋本　徹・八巻敏雄

ジベレリン $A_1 \cdot A_2 \cdot A_3 \cdot A_4$ の生理作用の強さをつぎの 3 種類の生理現象について比較した.

1. 明所で育てたイネ幼植物の第二葉の葉鞘の伸長生長.
2. 明所で育てたダイコンの第一葉の切片および暗所で育てたウズラ豆第一葉の切片の生長.
3. 発芽に光を必要とするタバコ種子の暗黒における発芽.

イネ葉鞘の伸長促進においては，GA_1 と GA_3 の強さがほとんど等しく，$GA_2 \cdot GA_4$ よりはるかにつよい. GA_4 は GA_2 よりやや強い. すなわち，$GA_1 \fallingdotseq GA_3 \gg GA_4 > GA_2$ で表わされるような強さの順序を示した. しかるに，ダイコンやウズラ豆の葉切片の生長を促進する作用は，GA_4 がもつとも強く，そのつぎに GA_3，つぎに GA_1 が続き（比較的低濃度では GA_3 より GA_1 の方がわずかに強い作用を示す），GA_2 は最も弱い. すなわち $GA_4 \gg GA_3 > GA_1 > GA_2$. タバコ種子の発芽でも GA_4 がもつとも強い作用を示し，そのつぎに GA_1, GA_3 が続き GA_2 はもつとも弱い. すなわちその効果の順序は $GA_4 \gg GA_1 > GA_3 \gg GA_2$ で表わされる.

イネの葉鞘の伸長促進における 4 種のジベレリンの強さの順序は，これまで報告されたチシャやヒメウィキョウの茎の伸長や花芽形成におよぼす促進作用の強さの順序と大体一致するが，葉の生長やタバコ種子の発芽では全く異なつている. 最も注目すべき差異は，イネ葉鞘の生長の場合には，非常に弱い促進作用しか示さなかつた GA_4 が，葉の生長やタバコ種子の発芽においては，他の 3 種のジベレリンにくらべてはるかに強い作用を示すことである.

このように 4 種のジベレリンの生理作用の強さの順位，特に GA_4 の順位は，ジベレリンによつて影響される生理現象の相違によつて異なることが示された.（東京大学教養学部生物学教室）

ベニシダ配偶体の形態分化と窒素化合物*

堀　田　康　雄**

Yasuo HOTTA**: Role of Nitrogenous Compounds in the Development of Gametophyte of *Dryopteris erythrosora*.*

1959 年 3 月 23 日受付

シダ類の配偶体形成，すなわち単細胞の胞子から成熟配偶体の完成（造精器・造卵器の完熟）にいたる過程は，種によって一定の形態的，生理的パターンを示す．しかし，同一種の同じ集団で生じたと思われる胞子集団を，同じような環境条件下で発芽させ，その後の発育過程をみると，かなりの形態的変異が認められる．光，温度，湿度，栄養などを適当に変化させた環境のもとで生育させた場合には，それに応じたさまざまな形態変化がおこることは古くから知られている[1])．

著者は，ベニシダを用いて配偶体分化を体内の物質変化の点より解析しようと試みた．

種々な形態分化のうち，1 列細胞よりなる線状体制の原糸体形成（一次元生長，*One-dimensional growth*）から一層細胞よりなる平面状体制の葉状前葉体（心臓形）形成（二次元生長，*Two-dimensional growth*）への転換を特に問題とした．この転換を二次元分化，*Two-dimensional differentiation* とよぶことにする（Fig. 1 においてⅡからⅢへの転換）．

さきに，われわれはベニシダ配偶体の二次元分化は体内の蛋白の質的変化を伴なうと予想される量的変化と密接な因果関係にあること[2])，さらに蛋白の変化はリボ核酸（RNA）の変化によるものであるらしいこと[3,4])，を報告した．

本報においては，主として二次元分化におよぼす窒素（N）化合物の影響および二次元分化に伴なう体内のアミノ酸の量的・質的変化をしらべ，すでに報じた二次元分化における蛋白の役割を，蛋白の素材の面より追究しようと試みた．

材料と方法

ベニシダ（*Dryopteris erythrosora*）を材料として用いた．胞子の発芽状態やその後の発育状態は，同一環境のもとにおいても，ときによって甚だしい変異がみられることがあるので，予備テストの結果，顕微鏡観察によってシャーレ内の約 75% の個体が同一の発育状態を示すような材料だけを実験に供した．

規準培養液として，少し組成を変更した Knop 液（KH_2PO_4 2.320 g，$Na_2HPO_4 \cdot 2H_2O$ 2.994 g，$Ca(NO_3)_2$ 0.2 g，KCl 0.024 g，$MgSO_4$ 0.05 g，$FeCl_3$ trace，蒸溜水 1000 ml）を用いた（Nagai[5]) はシダ配偶体の培養液として Knop 液がよいことを報じている）．いろいろな N 化合物の影響をみる場合には，この規準培養液の $Ca(NO_3)_2$ を目的とするN化合物と置換した．培養液は 7 日目または 10 日目ごとに更新した．

胞子の発芽およびその後の生長が可能な pH は 4.0～7.0 の間であり，しかも 5.1～5.6 が最適であることがわかった．これはベニシダの配偶体が成育する土壌の pH と大体同じであった．緩衝液として，燐酸，クエン酸，コハク酸，醋酸，ホウ酸を使用してみたが，燐酸がもっともよい．他のものではさまざまな異常が生じた．故に本報での実験は燐酸緩衝液で pH 5.3 に調整した培養液を用いた．培養は散光下（500～1000 Lux）27°で大型シャーレ（径約 12 cm.）中で液体培養法によって行なった．

配偶体の発生過程を，便宜上本報ではつぎの 7 時期に大わけして観察や測定の規準とした（Fig. 1）：Ⅰ 胞子，Ⅱ 5 細胞期に達した原糸体（一次元生長），Ⅲ 原糸体の先端細胞が分裂軸を 90° 回転し，平面葉状体ができはじめたとき，すなわち，二次元分化が起った直後の時期で 8～10 細胞からなる．二次元分化は規準培養条件では，7 細胞からなる原糸体の先端細胞で起る．Ⅳ 10～20 細胞からなる二次元生長期のもの．まだ分裂域（*Meristematic region*）は分化してない．Ⅴ 40～50 細胞の二次元生長期，

* 本報の一部は，日本植物学会第 20 回大会（広島・1955）において報告した．

** Biological Institute, Faculty of Science, University of Nagoya, Nagoya, Japan. 名古屋大学理学部生物学教室.

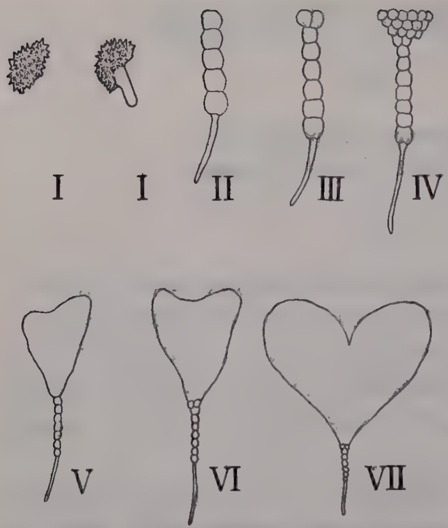


Fig. 1. Schematic representation of seven developmental stages of *Dryopteris erythrosora*.
I: Spore, I′: Germination, II: One-dimensional growth (5-cell stage), III: Two-dimensional differentiation, IV: Two-dimensional growth, V: Two-dimensional growth (Differentiation of meristematic region), VI: Two-dimensional growth (nearly mature), VII: Matured gametophyte.

分裂域は分化している. VI 二次元生長期の最終段階. VII 中肋部が多層細胞になりはじめ（三次元生長），ハート形配偶体の外形をとる時期.

上述の7時期について体内のアミノ酸の分析を行なった. 方法は Table 1 に示してある（大要は Steward *et al.*[6]）と同じである），非蛋白性部分のアミノ酸は 80% エタノール抽出物をペーパークロマトグラフにかけ分析した. 蛋白部分のアミノ酸は 80%エタノール抽出残渣を 6N HCl で加水分解し，二次元ペーパークロマトグラフ法で分析した. ペーパークロマトグラフィーに用いた溶媒は，ブタノール・醋酸・水（4：1：1），フェノール・水，ルチヂン・コリジンである. 更に，Dinitrofluorobenzene を用い，Isherwood and Cruickshank[7]）法にしたがつて spectrophotometrically に定量を行なった.

各時期で分析にかけた材料の一部をとってNの定量[8]）を行ないアミノ酸量を確認した.

結　果

1. N欠の場合　この培養条件では，規準培養液から $Ca(NO_3)_2$ を除いて培養すると，非常に弱い

Table 1. Preparation of the samples for chromatographic analysis.

```
ベニシダ配偶体(50 g)
  │
+80%エタノール(200～300 ml)抽出
  │ 5°C; 48～72 時間
遠心 2000×g, 5 min.
  ├─────────────────────┐
上 澄                   沈 澱
  │                   80%エタノール(200～300 ml)
  │                   で2回洗う（上と同じ操作）
  │                     ├──────────────┐
  │                   上 澄            沈 澱
  └─────────────────────┘          +6N HCl(50～100 ml)加水分解
  ├──────────┐                      オートクレーヴ 10 ポンド
減圧濃縮   減圧濃縮                     24～48時間
  │          │                          │
Nの定量    残 渣                      減圧濃縮
          +少量の蒸                     │
           溜水                  +1N NaOH(pH 6.3にする)
             │                          ├──────────┐
       (pH 6.3にする)               減圧濃縮     Nの定量
             │                    (pH 6.3にする)
       クロマトグラフ*                 クロマトグラフ*
```

* 東洋沪紙 No. 51. Phenol-water で展開する場合はあらかじめ pH 9.0の 0.1 M Borate buffer を吹きつけ乾燥したものを使用した.

一次元生長が続くのみで二次元分化は起らない. ただし，強い光（1200～1500 Lux）という条件にすると特殊な二次元分化と二次元生長がおこる. すなわち，一旦二次元分化するがそれ以上進まず，平面状に並んだ数個の細胞はそれぞれ一次元生長をするのでヘラ状の葉状体を形成する. 一見，二次元生長をしているようであるが，細胞分裂軸の方向からいって一次元生長をしているものの集まりにすぎない. なお Prantl[9]）は NO_3 欠の培地でシダ類配偶体が二次元生長をすることを報告している（ただし，分裂域の形成を阻止している）. これは，おそらく上述のような条件の場合であったのではないかと思われる.

2. N化合物を与えた場合　8種類の無機N化合物と 13 種類の有機N化合物をN源として与え，配偶体形成，とくに二次元分化におよぼす影響をしらべた. 各種類について，最も早く二次元分化をおこさせる濃度において，二次元分化に要する日数とそのときの原糸体の細胞数を Table 2 に一括して示す. この場合の規準はそれぞれ同一シャーレ内において二次元分化をおこした個体が 75% 以上であ

Table 2. Effects of some nitrogenous compounds on the two-dimensional differentiation of gametophyte.

Nitrogen-compounds used	Period from sowing to 2-dimensional differentiation in days	Cell numbers of gametophyte at the beginning of 2-dimensional growth
Standard: 1/5 Knop's soln. $Ca(NO_3)_2$	25	7
Control: N-free Knop's soln.	∞	∞
NH_4-salts: NH_4Cl, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, (NH_4)-citrate	14—24	5—10
NO_3-salts: $NaNO_3$, KNO_3, $Ca(NO_3)_2$	18—31	6—12
Amino acids and amides:		
a) Glycine, Serine, Valine, Cystine	18—21	5—6
b) Alanine, Leucine, Proline, Aspartic acid, Asparagine	25—31	7—8
c) Glutamic acid, Tryptophane	32—34	8—11

ることとした．N化合物の濃度は 10 mM，1 mM，0.5 mM，0.1 mM，0.05 mM，0.01 mM，0.005 mM，0.001 mM を規準とし，無機化合物の場合は 100 mM，80 mM，60 mM，50 mM，40 mM，30 mM，20 mM でも実験を行なった．

二次元分化を最も早くおこさせるN源としては NH_4Cl が有効であった．また，大略の傾向としては，アムモニウム塩が硝酸塩より有効であり，アミノ酸はその効果の大小から **a**＞**b**＞**c** の三群に分けられるといってよい (Table 2)．一般に，どの化合物も，高濃度ではいろいろな程度でさまざまの形態変異や生理的変異をおこす（発芽不能，発芽後の生育停止や死，クロロフィル減少，分裂方向の乱れ，分枝，集塊状の細胞集団形成など）．

与えたN化合物が，二次元分化以外の他の性質におよぼす変化のうち，特徴的なものをあげてみよう．NH_4Cl は原糸体細胞を球状にする傾向がある．$(NH_4)_2SO_4$ は低濃度において二次元生長を一次元生長へ戻すことがある．11.0 mM の KNO_3 で原糸体のクロロフィルは減少し，原糸体細胞は細長い形となり，さらに二次元生長に入った配偶体も細長い棍棒状となる．$NaNO_2$ は 0.015 mM 以下ではじめて規準培養液の場合と同じく働き，それ以上の濃度では極めて不規則な生長をもたらす．すなわち，硝酸塩に比べて低濃度で害作用を示し，決して促進的には働かない．Glycine, Proline, Glutamic acid, Tryptophane は，どの濃度でも原糸体にある程度の分枝をおこさせる．Glutamic acid は，そのほかに原糸体細胞を細長くさせる作用がある．

Table 3. Amino acids and amides of non-protein fraction obtained from gametophytes at various developmental stages (cf. Fig. 1). (The values in the table indicate the amount of the substances in micrograms obtained from every mg. of dry sample).

Amino acids	Stages I	II	III	IV	V	VI	VII
Leucine	0.6	0.6	0.6	0.6	1.0	1.0	2.0
Valine	0.6	0.6	0.6	0.8	2.0	2.0	3.0
Serine	?	?	?	0.5	0.5	?	?
Alanine	0.4	0.4	0.4	0.4	0.3	0.3	0.3
Glycine	0.6	0.6	0.6	0.6	1.0	1.0	0.9
Cystine	?	?	?	trace	3.0	5.0	5.0
Methionine	1.0	0.6	0.4	0.3	0.3	0.2	0.2
Aspartic acid	2.0	2.0	2.0	2.0	3.0	0.2	0.1
Asparagine	3.0	2.0	2.0	2.0	1.0	0.3	0.3
Glutamic acid	2.0	2.2	2.1	2.5	3.0	0.3	0.3
Glutamine	5.0	4.0	4.0	4.0	3.0	1.5	0.6
Tryptophane	0.0	0.0	0.0	0.0	0.0	0.2	15.0
Total	15.2	13.0	12.7	13.7	18.1	13.1	32.7

3. 配偶体内のアミノ酸類の分析

（i）遊離のアミノ酸およびアマイド．10 種のアミノ酸と 2 種のアマイドが検出され，配偶体形成の 7 時期についてそれぞれ semiquantitative な値が得られた (Table 3)．他の植物体には普通に見出される Proline, Hydroxyproline*, γ-Aminobutyric acid*, Phenylalanine および Cysteine* は検出されなかった．

* 分析方法が不完全であるため．

全量の変化でみると，二次元分化の前後（Ⅱ〜Ⅲ時期）にかけて僅かながら減少があり（専ら，Methionine, Asparagine, Glutamine の減少による），次には分裂域がはげしく形成される時期（Ⅵ）に再び減少がみられる（専ら Glutamic acid, Serine, Amides の減少による）．ハート形が完成する頃に全量は非常に増大する．

（ii） 蛋白部分から得たアミノ酸類　15 種のアミノ酸と 4 種の未知の Ninhydrine-positive spots を，配偶体の 5 時期から得た（Table 4）．ⅡとⅢの時期は材料の点と，数回の分析で得られた結果の再現性の点で完全なデータを得られなかったので表から省いた．（しかし，Unknown-3,-4 の点についてⅡでは，それぞれ 5 という明らかな値を得た）．Phenylalanine と Alanylglycine はどの時期においても存在していないといってよい結果である．用いた方法では，Hydroxyproline, γ-Aminobutyric acid，および Cysteine は検出されなかった．

全量の変化についてみると，前述の遊離アミノ酸類とはことなり，しらべた時期に関する限りⅥ期まで増加の一方であり，ハート形ができ上る頃に急減している．この点が遊離アミノ酸全量の変化の様子とはとくに著るしい対照をなしている． Arginine は二次元生長が盛んになるにつれ著るしく増大していること， Methionine は二次元生長のⅣ期に急激に増大していることが指摘される．

Table 4. Amino acids composition of protein fraction obtained from gametophytes at various developmental stages (cf. Fig. 1). (The values in the table indicate the amounts of the substances in micrograms obtained from every mg. of dry sample).

Amino acid	Stages						
	I	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ	Ⅶ
Leucine	3			23	12	12	?
Valine	trace			trace	trace	trace	5
Threonine	3			?	?	?	0
Serine	1			8	50	50	30
Alanine	?			10	15	24	24
Glycine	1			2	12	15	—
Cystine	12			12	12	—	—
Methionine	2			50	6	12	12
Aspartic acid	10			30	24	30	30
Glutamic acid	2			6	6	6	6
Arginine	12			15	120	180	150
Lysine	3			7	13	8	4
Tyrosine	8			15	6	6	6
Proline	3			?	4	?	0
Unknown 1	10			12	30	45	45
Unknown 2	10			12	15	15	15
Unknown 3	10	5		0	0	0	0
Unknown 4	10	5		0	0	0	0
Total	100	—	—	202	379	403	329

考　　察

1. 二次元分化と蛋白の質的変化　さきにわれわれは質的に異なる蛋白の急激な増加がベニシダの配偶体の二次元分化の必要条件である可能性を結論した[2,3,4]．しかし，この結論は (i) 質的に異なる RNA（ヌクレオチド組成が異なることから）の急増と二次元分化とが相伴なっている事実と (ii) 蛋白量の増大と二次元分化とが相伴なっている事実から導いたものである．

はたして，一次元生長期と二次元生長期で蛋白の質的ちがい，かつ二次元期で急増するのは二次元生長に特有な蛋白だけであるかどうかは現在行なっている実験に待たねばならない．

しかし，本報において行なった配偶体内のアミノ酸類の分析結果を上述の結論と一応結びつけて考えてみる．遊離アミノ酸類の質的，量的変化が二次元分化期にみられた．このことは何らかの蛋白の質的変化を意味するかもしれぬが，また同時にアミノ酸プール内だけのアミノ酸自身または相互の間の変化であるかもしれない．

ところで，蛋白部分から得たアミノ酸が二次元分化の前後で質的ならびに量的にも大きく変化しておれば，これは前報[2,3,4])の結論を有力に支持するものといえよう．ところが，二次元分化の前後の大事な時期（Ⅱ，Ⅲ）のデータが全く不充分であり断定的にはいえない状態である．しかし，二次元生長期のⅣ，Ⅴの結果をよく検討し，特に Leucine と Methionine が二次元分化後（Ⅳ期）に急激に増え，Unknown spots-3,4 は全く消失していることを指摘し，二次元分化の前後で蛋白の質（アミノ酸組成）が変化していることはまちがいないようで

ある．

なお，二次元分化以外の形態分化のことと関連していると思われる体内アミノ酸類の変化として，1) 分裂域形成のさかんなときに 2, 3 の遊離アミノ酸が減少すること（前述）と，2) 第Ⅳ期以後の蛋白の構成アミノ酸としての Arginine の増加は造精器，造卵器の形成と関連している(Nucleoprotein との関連）のではないか，ということを指摘しておく．

2. 体内への N 化合物のとりこみ N化合物を欠除した培地では二次元分化はおこらない．N化合物を与えると，たとえその作用が弱いとか多くの異常を示すとかいうことがあっても，ともかく二次元分化をおこす．この二つのことから，二次元分化がおこるためにはN化合物が外から体内へとりこまれることが必要である．胞子がもともともっていたN化合物は一次元生長だけに関連していると考えざるを得ない．(すなわち「一次元生長に特有な蛋白」というわれわれの考えの一助となる).

与えたN化合物の種類により，また用いた濃度により，二次元分化に対する影響はさまざまであった．それは，(i) 培地から原糸体細胞内へのN化合物のとりこまれかた，と (ii) 入つたN化合物の蛋白代謝パターンへのまきこまれかた，の相違によるわけであろう．

培地からまず仮根へ入り，仮根から原糸体の第1細胞 (Basal cell) へ移り，次第に先端細胞へとN化合物は移動していく（勿論，少しは直接，培地から原糸体細胞へ入る)．われわれが色素を用いてしらべたところ，外液から仮根へはどの色素も同速度で吸収されるが，仮根から原糸体細胞への移動は色素によつて速度が異なった．おそらく，N化合物の吸収の場合にもこのようなことがあるのではなかろうか．

アムモニウム塩の方が硝酸塩よりも，二次元分化をより速かにおこさせる．また同じ程度で二次元分化がおこされる場合でも，アムモニウム塩の方が遙かに低い濃度で有効である．このことは，アミノ酸の合成過程での代謝パターンにアムモニウムが関係している，ということから説明しうるであろう．Nagai[5] はアムモニウム塩で培養すると配偶体は死ぬ，と報告しているが，これは濃度が高すぎるためであると思われる．亜硝酸塩は硝酸塩よりも二次元分化に対して悪効果（遅らせたり，異常形態をとらせたり）を与えているが，これは一般に種子植物のN代謝パターンにおいて NO_3, NO_2 の還元系，酸化系が重要であることから理解されよう．

アミノ酸類を与えた場合，二次元分化に対する効果はさまざまであったが，それは上述の場合と同じく，アミノ酸の種類によつて吸収程度に差があるとか，二次元分化に特有な蛋白質へ組みこまれる程度が異なるということが考えられる．この点については，本報の実験では確かなことは論じられない．なお，Sossountzov[10] は，*Gymnogramme* において Glycine は二次元生長をうまくおこなえないとか，Leucine, Serine, Alanine は Knop 液よりも二次元生長に対する効果が悪いと報じている．われわれのベニシダの場合の結果と併せ考えてみると配偶体の形態分化の機構を考察する上で興味がある．

本研究について，御指導御鞭撻下さった島村環，原田市太郎両博士に感謝する．

引 用 文 献

1) Klebs, G., Sitzber. Heidel. Akad. Wiss., Math. Natur. Kl. B, Teil I, II, III. (1917). 2) Hotta, Y. and Osawa, S., Expl. Cell Res. **15** : 85 (1958). 3) 堀田康雄 第 23 回日本植物学会大会. (1958), 4) Hotta, Y., Osawa, S. and Sakaki, T., Develop. Biol. In press. 5) Nagai. I., Flora (Jena) **106** : 281 (1914). 6) Steward, F, C., Wetmore, R. H., Thompson, J. F. and Nitch, J. P., Amer. J. Bot. **41** : 123 (1954). 7) Isherwood, F. A. and Cruickshank, F. A., Nature **174** : 123 (1954). 8) Yagi, Y., Nucleic acids and Nucleoproteins, I : 132., ed, by Egami, *et al.*(1951). 9) Prantl, K., Bot. Zeit. **39** : 753 (1881). 10) Sossountzov, I., Croissance, sexualité et dimensions des prothalles de *Gymnogramme calomelanos* en culture aseptique sur quelques milieux azotes mineraux. Thèses. (La Faculté des Sciences de l'Université de Paris).

Summary

1. Effect of nitrogenous compounds on the morphological differentiation of gametophyte of a fern, *Dryopteris erythrosora*, was investigated. Studies were carried out for the most part about the effects of nitrogenous compounds on "the two-dimensional differentiation" (conversion of the filamentous organization to the plate-like one).

(i) In the nitrogen deficient culture the two-dimensional differentiation does not occur.

(ii) NH_4–salts are more effective than NO_3–salts for the induction of the two-dimensional differentiation. $NaNO_3$ reveals many deleterious effects.

(iii) Several amino acids, which were used in this experiment, are classified in the following three groups according to their effects on the two-dimensional differentiation: a) those which induce the differentiation earlier than in the standard culture, b) later than in the standard, and c) the same as in the standard.

2. Analyses of amino acids both in the alcohol soluble fraction and in residual fraction were carried out at various stages of the gametophyte differentiation. Ten amino acids and two amides were detected in the alcohol soluble fraction; some of them showed characteristic changes in their amounts corresponded to the development of the morphological differentiation. Twenty amino acids including 4 substances of unidentified nature were detected in the residual fraction; significance of the change in their amount is discussed in connection with the two-dimensional differentiation.

3. The author's view (1958) that the qualitative change of protein may be responsible for the induction of the two-dimensional differentiation, is also supported by the results of the present experiments.

ヒロメ，アントクメおよびカジメの胞子嚢群の発生について（予報）*

西林長朗**・猪野俊平**

Takeo NISHIBAYASHI** and Shumpei INOH**: On the Sorus Development in *Undaria undarioides* (Yendo) Okamura, *Eckloniopsis radicosa* (Kjellman) Okamura and *Ecklonia cava* Kjellman. (Preliminary Note)*

1959 年 8 月 15 日受付

ヒロメ（*Undaria undarioides* (Yendo) Okamura)，アントクメ (*Eckloniopsis radicosa* (Kjellman) Okamura) およびカジメ (*Ecklonia cava* Kjellman) の3種はともにコンブ目植物に属している．コンブ目植物の胞子嚢群は遊走子嚢と，生殖に関係のない単細胞の側糸とが互いに柵状に並んでできている．このような胞子嚢群の発生について，従来，多くの研究[1,2,3,4,5,6,7,8]がなされてきたが，邦産のものについては報告がない．それ故，著者ら[9]はわが国に産するツルモ，マコンブ，ワカメおよびチガイソについて観察を行なった結果，ツルモ，マコンブおよびチガイソの3種はそれぞれ胞子嚢群の発生様式が異なっているということを見出した．今回，前研究に引きつづきヒロメ，アントクメ，カジメの3種について観察を行なったところ，また新らしい知見が得られたので，それをここに予報する．

材料と方法

本研究に用いた材料は，ヒロメ（*Undaria undarioides* (Yendo) Okamura)，アントクメ（*Eckloniopsis radicosa* (Kjellman) Okamura)，カジメ (*Ecklonia cava* Kjellman) の3種であり，ヒロメは 1958 年3月に和歌山県田辺湾で，アントクメおよびカジメは 1958 年8月に静岡県下田町の海岸にて採集したものである．採集後，胞子嚢群をつけた個体を選び出し，この部分を細かく切って阿部氏液[10]で固定した．固定時間は 15～24 時間である．固定後は普通のパラフィン切片法により 5～6 μ の切片をつくり，10％過酸化水素水で漂白した後，ハイデンハイン氏鉄明礬ヘマトキシリンで染色しプレパラートを作成して観察を行なった．

観　　察

1. ヒロメ (*Undaria undarioides* (Yendo) Okamura)

著者らが採集したヒロメでは，ワカメのような，柄の両側に波状に屈曲した胞子葉をつけた個体は1個体もなく，すべて胞子嚢群は普通の葉の両面につくられる．胞子嚢群は最初，葉の中央部の中肋の両側からでき始め，その後，葉の下方に向かって形成が進み，遂には葉の下方，全面にわたってつくられるようになる．胞子嚢群の形成は葉の両面で，平行してほとんど同時的に行なわれる．

葉はその両面に細胞が1層に規則正しく並び，色素体を含んだ表層と，色素体をほとんど持たない皮層と，糸状細胞よりなる髄の3部よりなっている．成熟期に入ると，1核と数箇の色素体を含んでいる表層細胞は，その細胞の一つずつが葉面に平行な膜により，外側の上位細胞と内側の下位細胞とに各々分けられる (Figs. 1, 2)．上位細胞は伸長して，そのまま生殖に関係のない単細胞側糸となる．一方，下位細胞は伸長した上位細胞の間に突出し始める (Figs. 3, 4)．同時に，下位細胞の核は分裂して2核となる．その中の1核は 2～3 個の色素体とともに突起に向かって移動し，これらが突起の中に入り込んだ時，突起は隔膜によって下位細胞から仕切られて遊走子母細胞となる (Figs. 5, 6)．その後，遊

---

* 文部省科学研究費，課題番号 407127
岡山大学理学部生物学教室植物形態学研究業績 No. 72
玉野臨海実験所業績 No. 54

** 岡山大学理学部生物学教室 Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.

Figs. 1–7. Development of sorus of *Undaria undarioides* (Yendo) Okamura. All magnifications ca. × 850.
Fig. 1. Section of lamina in sterile portion, showing one layered meristoderm. Fig. 2. Transverse division of the meristoderm into the lower cell and upper cell (future paraphysis). Fig. 3. Growth of the upper cell. Fig. 4. Projection of the lower cell between the adjacent young paraphyses, and nuclear division in the lower cell. Fig. 5. Migration of a nucleus with two or three chromatophores towards the projection. Fig. 6. Transverse division of the lower cell to form the zoospore-mother-cell. Fig. 7. Further stage of development.

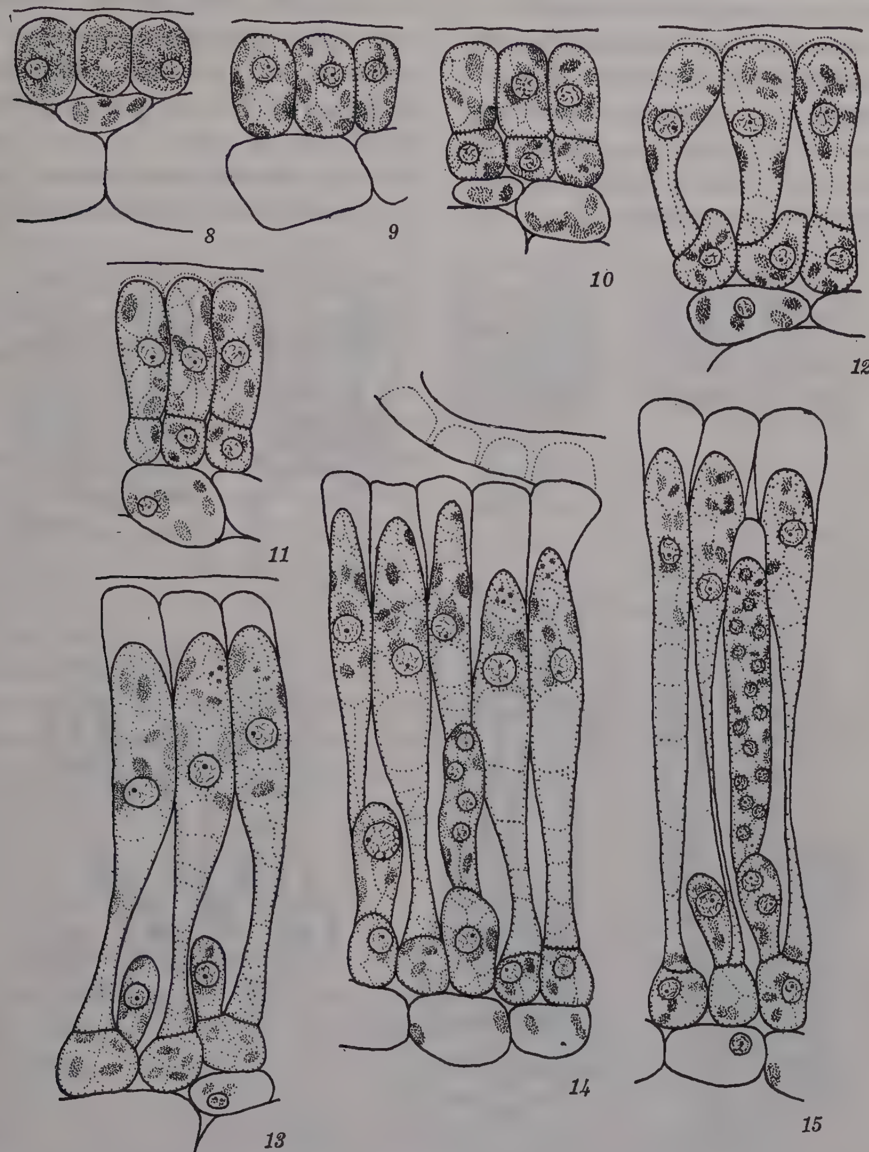
走子母細胞の生長にともなって，側糸は伸長して，その頭部は大きくなるが，基部は徐々に細くなり糸状となる．一つの遊走子母細胞がつくられると，下位細胞は再び別の遊走子母細胞を切り出し，かくして一つの下位細胞から 2～3 の遊走子母細胞が形成される．完成した側糸の頭部は三角形状で，その中には 1 核と数箇の色素体が含まれている．側糸の外膜は肥厚しているが，その肥厚は側膜にまでおよんでいない．側糸の外側は薄い粘液角皮で覆われている (Fig. 7).

2. アントクメ (*Eckloniopsis radicosa* (Kjellman) Okamura)

アントクメの胞子嚢群は，葉の裏面の基部の方からでき始め，次第に上部に向かって形成される．後には葉の表面にもつくられるようになる．

1 層に整然と並んでいる表層細胞には，細胞の全内容をほとんど占有するような一つの色素体が含まれ，その布状にひろがつた色素体にとりかこまれて 1 個の核が存在する (**Fig. 8**)．成熟期に入ると，この布状にかたまった色素体は細分されて，他のコンブ目植物で見られるのと同じような，散在した数箇の楕円体状の色素体となる (**Fig. 9**)．このように色素体に変化が起った表層細胞は，間もなく横裂して，将来，単細胞側糸に生長する外側の上位細胞と内側の下位細胞とに分けられる (**Fig. 10**)．上位細胞が伸長して細長くなったとき，下位細胞はこれら

の上位細胞の間に突起を出し，突起がある程度大きくなると，突起は隔膜によって下位細胞から仕切られて，これが遊走子母細胞となる (Figs. 11, 12, 13). この頃から側糸の外膜は肥厚してくる．側糸は伸長にともなって，その基部はいくらか細くなっていく．遊走子母細胞が生長し，その核が分裂を始



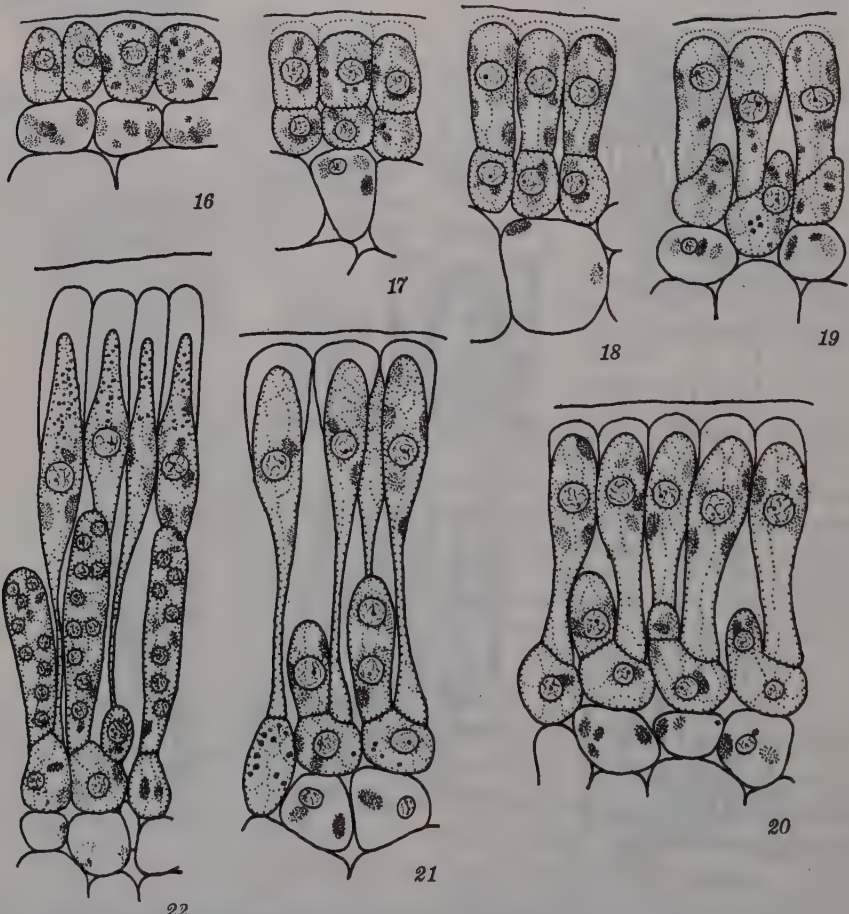
Figs. 8–15. Development of sorus of *Eckloniopsis radicosa* (Kjellman) Okamura. All magnifications ca. ×850.

Fig. 8. One layered meristoderm in sterile portion. Fig. 9. Meristoderm containing a nucleus and some chromatophores. Fig. 10. Transverse division of the meristoderm to form the lower cell and future paraphysis. Fig. 11. Growth of the paraphyses. Fig. 12. Projection of the lower cell between young paraphyses. Fig. 13. Transverse division of the lower cell to form the zoospore-mother-cell. Fig. 14. Separation of the cuticle from the paraphyses. Fig. 15. Further stage of development.

めて2核となり，さらにもう一度の引きつづきの核分裂によって若い遊走子嚢内に4核がつくられる頃になると，側糸の外側を覆っていた角皮が剝離してきて，側糸は互いに離れ易くなり，その後は外界の影響を受け易い状態で遊走子嚢は生長する (Fig. 14). 胞子嚢群の形成が急速に進んでいるところでは，表層細胞の二分によって生じた上位細胞の伸長が著るしい．それ故，角皮はこの上位細胞の急速な生長にともなって，伸展することができないで，上位細胞が伸長し始めた時に剝離してしまうことがある．一つの遊走子母細胞が大きくなると，下位細胞は再び別の遊走子母細胞を切り出し，かくして一つの下位細胞から，二つの遊走子母細胞が形成される．完成した側糸の頭部は棍棒状で，その中央に1核を含み，核の周囲には数箇の色素体が散在している (Fig. 15). アントクメの遊走子嚢は非常に細長く，互いにくっつき合っている側糸の頭部の間に入り込んでいる．

3. カジメ (*Ecklonia cava* Kjellman)

カジメの胞子嚢群は，最初，葉片の各所に円い斑



Figs. 16–22. Development of sorus of *Ecklonia cava* Kjellman. All magnifications ca. × 850.

Fig. 16. One layered meristoderm. Fig. 17. Transverse division of the meristoderm to form the lower cell and future paraphysis. Fig. 18. Growth of the paraphyses. Fig. 19. Projection of the lower cell between young paraphyses. Fig. 20. Migration of a nucleus with one or two chromatophores towards the projection, and transverse division of the lower cell to form the zoospore-mother-cell. Figs. 21, 22. Further stages of development.

紋となって生じるが，後には全葉面に拡がり，葉の両面につくられる．

1層に整然と並んだ葉の両面の表層細胞には，1核と，そのまわりに球形または楕円体状のいくらかの色素体が含まれている (Fig. 16). 成熟期になると，この表層細胞に変化が生じる．表層細胞は葉面に平行に走る膜により，外側の上位細胞と内側の下位細胞とに分けられる (Fig. 17). 上位細胞はそのまま伸長して単細胞側糸となる．上位細胞が幾分か伸長して細長くなったとき，下位細胞はこれらの若い側糸の間に突起を出す (Figs. 18, 19). 同時に下位細胞の核は分裂して2核となるが，その中の1核は下位細胞の中央部に留まり，他の1核は1個または2個の色素体とともに突起の方へ移動する．核および色素体が突起の中へ入ってしまった後に，突起は隔膜によって切り出されて，これが遊走子母細胞となる (Fig. 20). この頃から側糸の外膜は肥厚してくる．側糸は生長するにつれて，基部は次第に細くなって糸状となり，その間で遊走子嚢は生長する (Fig. 21). 一つの下位細胞から，二つの遊走子嚢がつくられている場合がしばしば観察された．でき上った側糸の頭部は棍棒状を呈し，その中央に一つの核が，核の周囲には色素体および同化産物と思われる多数の小さい顆粒が存在する (Fig. 22). 側糸の外側は角皮で保護されているが，角皮は遊走子が成熟するまで存在し，アントクメのように発生の途中で消失することはない．

考　　察

以上の観察結果から，ヒロメ，アントクメおよびカジメの胞子嚢群は，葉の表層細胞から発生することが判る．著者ら[9])は前に，コンブ目植物の胞子嚢群の発生様式には，三つの型があることを報告した．その一つはマコンブなどで見られるもので，表層細胞の上下二分によって生じた上位細胞は，そのまま伸長して単細胞側糸になるが，下位細胞は側糸の間に突起を出し，この突起が隔膜によって仕切られて遊走子母細胞になる（マコンブ型). 第二の型は，チガイソで見られるもので，上位細胞はマコンブ型のものと同じように側糸になるが，下位細胞からの突起は遊走子母細胞とならないで，これもまた側糸となり，その後，下位細胞から改めて突起を生じ，この2回目の突起が遊走子母細胞となる（チガイソ型). 第三の型はツルモがとる発生の様式で，上位細胞は側糸となり，下位細胞から生じた突起は直ちに遊走子母細胞になることは，マコンブと同じであるが，表層細胞の外側を覆っていた角皮は，発生の初期に消失する．このため，遊走子嚢はばらばらになった側糸の間で生長する (ツルモ型).

ヒロメおよびカジメの両種は，この三つの発生様式のうち，最初の型，すなわちマコンブ型に入れられるが，アントクメでは遊走子母細胞核が分裂して，若い遊走子嚢内に4核がつくられる頃までに，胞子嚢群を保護していた角皮は完全にはがれてしまい，側糸は互いに離れ易い状態となる．このようにアントクメが，遊走子嚢の発生の途中で角皮が消失するという点では，ツルモと同じであり，ツルモ型に入れられる．

神田 (1939)[11]) はアントクメの遊走子嚢が仮根にまで形成され，このような性質は他のコンブ目植物で見られない特異な性質であると述べている．アントクメのこのような性質と，遊走子嚢の発生の途中で角皮がはがれて消失するという性質とを思い合わせて考えると，アントクメはツルモに近い種ではないかと思われる．またアントクメの未成熟の葉の表層細胞には，細胞のほとんど全内容を占める布状の一つの色素体が存在するが，このこともまたアントクメに見られる特異な性質である．

ヒロメとカジメでは側糸細胞の形に差異があって，カジメの側糸の頭部は棍棒状であるが，その外膜の肥厚はマコンブほど著るしくなく，著者らがすでに報告したスジメ (1957)[12]) に類似している．ヒロメの側糸の頭部は三角形状を呈し，ワカメの側糸にその形が非常によく似ている．著者らが観察したヒロメには，ワカメで見られるような柄の両側につくられる特別な胞子葉はなく，胞子嚢群は普通の葉の両面につくられる．このように，ヒロメとワカメでは胞子嚢群がつくられる位置が異なっているけれども，その側糸の形は非常によく似ている．

本研究を行なうに当り，材料の採集および実験に多くの便宜をお計り下さつた，東京教育大学附属下田臨海実験所所長高槻俊一博士，ならびに千原光雄氏，および和歌山県水産試験場の所員の方々に厚くお礼申し上げます．

文 献

1) Thuret, G., Ann. Sci. Nat., Bot. 3, **14** : 214 (1850). 2) Setchell, W. A., Proc. Amer. Acad. Arts and Sci. **26** : 177 (1891). 3) Sauvageau, C., Mém. Acad. Sci. Paris **56** (1918). 4) Kylin, H., Svensk Bot. Tidskr. **12** : 1 (1918). 5) McKay, H. H., Univ. California Publ. Bot. **17** : 111 (1933). 6) Herbst, C. C. and Johnstone, G. R., Bot. Gaz. **99** : 339 (1937). 7) Clare, T. S. and Herbst, C. C., Amer. Jour. Bot. **25** : 494 (1938). 8) Hollenberg, G. J., Amer. Jour. Bot. **26** : 34 (1939). 9) Nishibayashi, T. and Inoh, S., Biol. Jour. Okayama Univ. **4** : 67 (1958). 10) Abe, K., Sci. Rep. Tohoku Imp. Univ., Biol. **8** : 259 (1933). 11) 神田千代一, 植雑, **53** : 271 (1939). 12) Nishibayashi, T. and Inoh, S., Biol. Jour. Okayama Univ. **3** : 169 (1957).

Summary

The development of sorus of *Undaria undarioides* (Yendo) Okamura, *Eckloniopsis radicosa* (Kjellman) Okamura and *Ecklonia cava* Kjellman has been observed. In all of three species both the zoosporangia and paraphyses originate from the meristoderm of the blade. In *Undaria undarioides* and *Ecklonia cava*, the mode of the development of sorus is *Laminaria*-type. In *Eckloniopsis radicosa*, the cuticle of the superficial cells is stripped on the way of sorus development. This fact seems to suggest that *Eckloniopsis radicosa* is identical in the mode of sorus development with *Chorda filum*.

Dry-Matter Reproduction in Plants 1.
Schemata of Dry-Matter Reproduction

by Masami MONSI*

Received August 11, 1959

Dry-matter production is the key function in ecological and sociological life of plants (Boysen Jensen[1]), Monsi and Saeki[2])), whether they grow singly or constitute a closed plant community. Hence, in the field of the modern social science of plant which originated from Boysen Jensen's classical work, Die Stoffproduktion der Pflanzen, 1932[1]), a number of workers in Denmark[3–10]), Japan[2, 11–27]) and other countries[28, 29]) have already made many studies and are securing some precise information on the dry-matter production in plants and plant communities. Recent works[30–34]) on growth analysis of the English school should also be noticed here. Moreover, in the general ecology whose final subject is the ecosystem which can maintain itself only on the basis of the primary production by plants, the latter is no doubt the primaries to be studied[35, 36]).

The dry-matter production cannot be maintained without "reproduction" of matter. In order that the plant continues to develop, the matter produced by photosynthetic system in a production period must always be transformed into the production system or a part of it in the successive period.

With regard to this problem, however, have been presented so far only three forms of balance sheets of annual dry-matter production by Boysen Jensen[1,37,38]) (cf. also 6–10,28) and a chart of distribution of product in Baker's textbook[39]), but no general schemata of dry-matter reproduction in plants. The balance sheet indicates statical features of the prodution, and it may hardly elucidate the dynamic aspect of development of plants and plant communities. The latter problem, which is also fundamental to a true comprehension of social relationships between plants, should be clarified on the basis of the dry-matter reproduction.

In this paper the author will present major types of schemata for dry-matter reprodution systems and make a practical application of these schemata to a comparative study on the shade tolerance of *Pinus* and *Picea*[40]).

General features of dry-matter reproduction in plants

The plant E (whether individual or community) can be divided into two main components[2]), photosynthetic system F and non-photosynthetic C; the former generally performs its function in photosynthesis as well as in interception of light as leaf "area" $\bar{F}$, and the latter, according to the subject, should be classified into stem C_H and roots C_W, and sometimes in addition branches and twigs C_B. In the production process, only the photosynthetic system works as the producer of surplus product. The quantity (F), photosynthetic rate a and respiration rate r of the photosynthetic system determine the gross production $P_g=F\cdot a$ (after Boysen Jensen 1946[38])) and "surplus" production $P_s=F(a-r)$ in the plant. The relation of P_g and P_s to the amount of F has been demonstrated in previous papers[2, 11]) (see also 34,41,42), and the results are that the maximum production of a closed plant community with thick

* Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

foliage is mainly determined by impinging light intensity; in other words, with mutual shading of leaves, further increase of F beyond a certain amount brings about no increment of P_s, and the amount of F of the community is also fixed by the light intensity, as the leaves of the bottom of the community should sooner or later perish in the illumination below their compensation point. — There is an optimum amount of F for the P_s maximum in the plant community. Moreover, the productivity is determined by photosynthetic hours (deciduous or evergreen, day length, duration of stomatal opening — here water economy must be considered[43]) and diurnal and annual temperature courses and by variation of photosynthetic rate with leaf age[44, 45]) and nutrient salts[3,4,46,47]). The simple term $F\ (a-r)$, therefore, really implicates very complicated functions.

Out of P_s a part must be spent for respiration R in the C, where $R=C\cdot r_c$, and the residue of P_s-R is the net production P_n of the plant. The P_n corresponds to the matter to be used for formation of new tissues of photosynthetic ΔF and of non-photosynthetic system ΔC. These newly built tissues make a part of each system in the next production period together with the already existing system (cf. Iwaki[22, 23])) and thus the "expanded" reproduction is assured. In the case of transformation from raw material into completed tissues, some loss in matter occurs because of shedding-off of e.g. bud scales, and of especially high respiration for growth. The ratio in dry weight of completed tissues to raw material is designated here as "transformation factor", which corresponds to Midorikawa's "economic ratio"[25]). The value was about 0.5–0.6 in the case of development of young plant from tuber in *Aconitum*[25]), potato[25]) and *Helianthus tuberosus*[27]).

An individual plant develops in general from a seed S, and the seedling produces dry-matter under given conditions of its habitat. The plant or production system grows and matures with repeating its production of matter by photosynthesis and the transformation of the product into F and C. After several production periods, flowers B appear and the fructification occurs. Thus the first generation started from one seed finishes in several or generally innumerable seeds (mS), and the second generation will begin at a great number of young plants $m'E$, if the plant species is possessed of Vitality 1[48]) in the given environment.

$$S \dashrightarrow E \left\{ \begin{array}{l} F \longrightarrow \\ \qquad \searrow \quad +P_n \\ C \longrightarrow \end{array} \right\} \begin{array}{l} +\Delta F + + + \sum F \\ \\ +\Delta C + + + \sum C \end{array}$$

$$B \dashrightarrow mS \dashrightarrow m'E \left\{ \begin{array}{l} m'F \\ m'C \end{array} \right.$$

Types of dry-matter reproduction

As for representative life forms the features in reproduction above-mentioned should be discussed more in detail. The dry-matter reproduction actually proceeding in the plant, however, is so intricate that the discussion must be limited only on abstracted fundamental characteristics. The general schemata are summarized in Table 1.

I. Herb systems. These are generally distinguished from tree systems by the fresh, living non-photosynthetic system ($C=L$) which is relatively small in size and short in duration.

1. *Annual herb system* (therophytes after Raunkiaer[49]). Characteristics of this system are seed formation in a shorter production period. The C as well as the F dies soon after finishing production process, leaving only seeds behind. Thus, the succession of generation is annually perfomed through seeds. In this case multiplication of population occurs normally. Numbers of new seeds mS develop in the next year to a certain number of plants $m'E$. The number of the latter m' is fairly smaller than that of the former m, as the seeds and seedlings suffer damages from fungi, insects, birds, rodents, etc. Nevertheless it is sufficiently large for rapid multiplication of plant individuals, and this can give rise to a prompt increase of production centres. Therefore, this system is the fittest as the initial vegetation with rapid expansion of matter production in plant succession on bare land. When the site area is large enough, the second year's production is m'-times as large as the first year's, and $(m')^2$-multiplication should occur in the third year. In the vegetation of a limited area, however, this gives rise to overpopulation very shortly and the development of each constituent is restricted by intraspecific competition[22, 50–52]. As discussed already, the optimum amount of F is definite in a plant community[2,11,34,41,42], the overpopulation thus will bring reduction in P_n on account of the depression in a and relative increase in C[22]. Therefore, if the site area is limited, overpopulation eventually causes "reduced" reproduction and consequently the ruin of the community. Here occurs the plant succession or the change of reproduction systems.

"Simple" reproduction will be maintained in this system under the condition of $m'=1$. The highest yield with an optimum population can continuously be secured by artificial control of sown seeds in number, i.e. by harvesting of $(m-1)$ seeds from the field. This is the principle of cereal or crop cultivation.

To conduct more detailed investigations into the successive growth of plants, the production and transformation processes should be studied in a proper, rather much shorter production term than one year which is usually adopted as one production period in case of discussion on natural vegetation and yield of field crops, etc. The production term should be here one month, or one week or sometimes even one day. In such a short production term, F and C remain even in annual plants without dying, ΔF and ΔC being successively accumulated to respective systems (cf. Iwaki[23]) p. 133). In this case the schema for annuals becomes quite similar to that for the long-term vegetative growth of the permanent herb and tree systems without defoliation (cf. the following dicussion).

2. *Perennial herb system* (cryptophytes and hemicryptophytes). This system is the same as the annual herb system in regard to the fact that not only F but also C is dried up after annual production. In this point these herb systems are opposed to the tree system whose C remains over a long period of time. Nevertheless there is a critical difference between annual and perennial herbs in the mode of continuation of dry-matter production in the successive years. The former make seeds for the next generation, while the latter continue their production mainly through tuber or rhizome G, although there can be recognized no substantial difference between these organs as to the function as latent organs which pass the hard season below or in the soil-surface. — For example, large, nutrient rich seeds of *Castanea*, *Crinum* and some legumes are ecologically almost the same as tubers, and on the contrary numberless small bulbs of *Oxalis martiana* are the same as ordinary seeds. It may, however, be generalized that in annuals the final result of production reveals itself in the population increase, but in perennials, in increment in size of each individual. Therefore, in the initial phase of plant succession, perennial plants cannot

increase in general their matter production so rapidly as annuals do, because the production centres do not increase in the former as in the annuals that can propagate in the manner of geometric progression. After seveal years, however, the tuber, and hence the matured perennial plant can grow up in a quite large size with accumulation of assimilates, as observed e.g. in altherbosa by Midorikawa[25]). By means of tall and vigorous growth on the cost of reserved matters the perennial plant eventually defeats the annuals in interspecific competition, — concerning the importance of growth in height to interspecific competition, refer Boysen Jensen[9]), Iwaki[23]). As a result of this the prennial plant community usualy succeeds in plant succession the pioneer community of annuals[53]).

In a closed mature community, F and consequently P_s are almost constant as mentioned above. In this case, if C and thereby R are constant, also $P_s-R=P_n=G$ will be constant, because in this herb system the C/F ratio[22]) can remain in a constant value year by year with the renewal of the whole production system. This means $F_1=F_2=\cdots=F_x$ and $C_1=C_2=\cdots=C_x$, and there is no contradiction in the repetition of "simple" reproduction. In practice this is proved by high possibility of subclimax of herbosa constituted by geophytes and hemicryptophytes[13, 25]).

3. *Permanent herb system* (including some herbaceous phanerophytes). A few of herbs, such as *Ophiopogon*, *Liriope*, *Aspidistra*, *Rhodea*, have perennial evergreen leaves. In those herbs, also C remains in a long time without perishing. Consequently the P_n can solely be distributed into ΔF and ΔC, without supplementing the dead parts of the production system. The increase and maintenance of F must be rapider and easier in this system than in the foregoing two systems, because of lacking in defoliation, provided that other conditions are the same. Therefore, this system is apparently favourable for production in case of small P_s, e.g. under deep shade of forest. However, if $\Delta C/\Delta F$ is large (in reality rather poor C_H is observed in the said species) and C increases continuously, the R also increases rapidly and endlessly, as the whole C remains fresh and alive so far the plant grows. Accordingly, $P_s-R=P_n$ becomes smaller and smaller with plant growth, because P_s is constant in a closed plant community, and eventually the P_n will turn zero and the plant should remain without growth, or, if the plant continues further to increase its non-photosynthetic system, the plant must reduce the production with reduction of the photosynthetic system.

This contradiction in the permanent herb system is avoidable to a great extent by saving the dry-matter consumed in respiration by C with converting a part of living tissue L into non-living productive tissue D, as noticed in tall and large trunk of the tree. Such a production system is no more belonging to the herb system, but to a tree system.

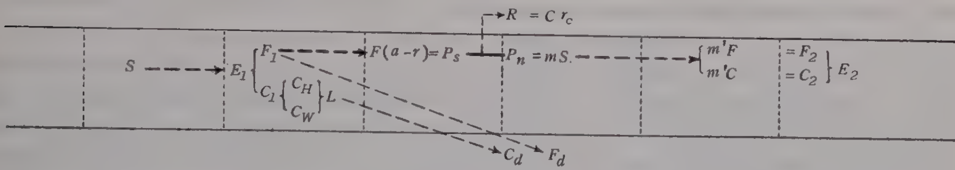
II. Tree systems (chamaephytes and phanerophytes). Besides the above discussed characteristics that a part of L, especially in tree trunk, changes itself into D in or after production process, here should be added some specialities, e.g. the storage of substance which can be mobilized in any time and the development of branches and twigs C_B. Although the amount of loss by shedding-off of C_B is fairly large[1,6,7,10,14–16]), this is excluded from the discussion of production schemata for the sake of simplicity, and the essential may not be changed by the omission of the amount. The storage of assimilates is observed also in herb system, particularly in tubers or rhizomes of perennial herbs. In the case of tree systems, the P_n is stored in the C as the preparation for supplement or development of the production system in the next year, and some part of the storage will continuously remain there as mobilizable substance

Table 1. Schemata of dry-matter reproduction in plants. Explanations of the symbols are in the text.

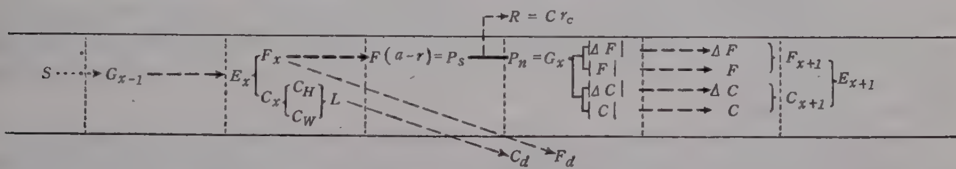
	Transformation process	Production system	Production process	Yield	Transformation process	Production system

A. HERB SYSTEM

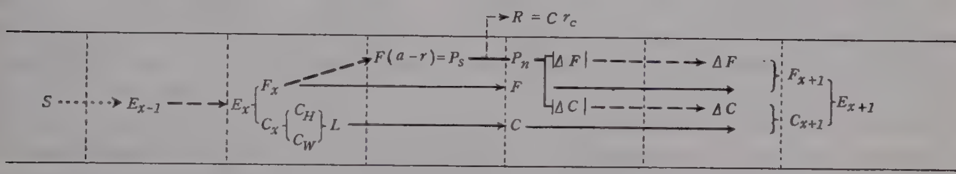
1. *Annual herb system*



2. *Perennial herb system*

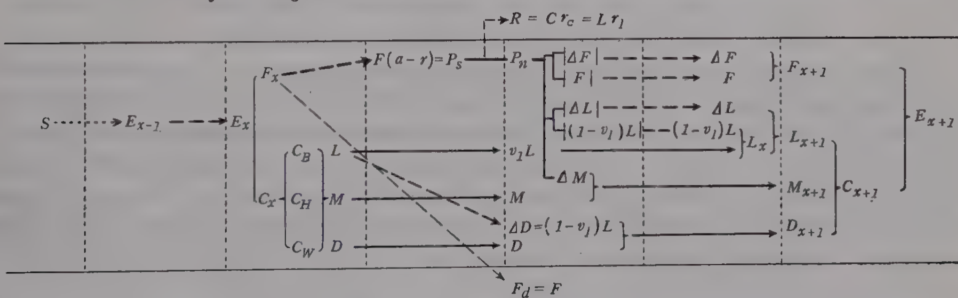


3. *Permanent herb system*

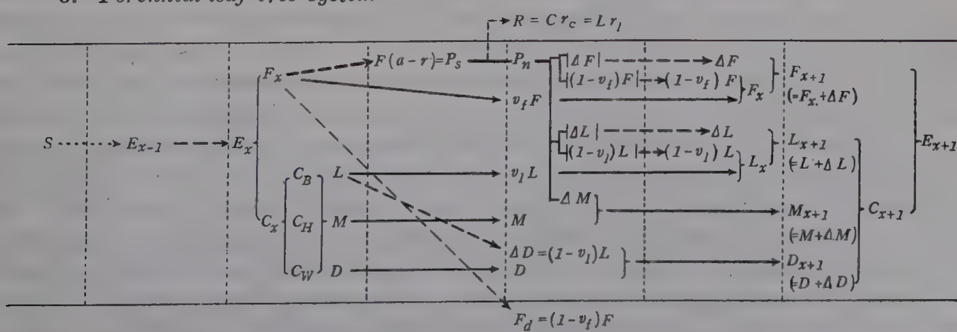


B. TREE SYSTEM

4. *Annual-leaf tree system*



5. *Perennial-leaf tree system*



M to complete the fructification and to supplement the production system when it is injured by any cause (cf. Midorikawa[25])).

Two cardinal types are recognizable concerning the life duration of leaves.

4. *Annual-leaf tree system.* As to longevity of leaves two categories, i.e. deciduous and evergreen, are so far usually distinguished. This classification, however, is not sufficient for the discussion of dry-matter reproduction, because some evergreen trees, mostly broad-leaved ones, shed all leaves just after unfolding of new leaves, moreover some, e.g. *Cyclobalanopsis glauca* in Tokyo, are even bare in a short period just before coming of new shoots. Leaves of most of broad-leaved evergreen trees fall after an annual production period. Therefore, the whole photosynthetic system of the next year should be prepared every year to continue the matter production. The difference between deciduous tree and such kind of evergreen tree is in the time length of matter production, especially in warmer climate. If it is severely cold, however, the matter production in evergreen trees in winter attains to a small, sometimes negligible, amount at most[19,21,29,54]). So in the region with cold winter, little difference between evergreen trees and deciduous ones can be recognized concerning the total amount of annual production.

5. *Perennial-leaf tree system.* Many coniferous trees and some broad-leaved evergreen trees (and shrubs) have perennial leaves[55]). The photosynthetic activity of leaves of several years old was proved by Stålfelt[56]), Hiramatu[57]), and Kuroiwa[58]). In this production system the annual renewal of F occurs only partly, so the dry matter reproduction can continue at the expense of a small part of P_n for the maintenance of F. This characteristic is much favourable for plant life in the same way as discussed in the case of the permanent herb system. When the matter production is inhibited by some environmental conditions, especially by weak illumination or cold temperature (alpine or subarctic), long-lived leaves become essential for survival of plants, as observed in a palm *Livistona subglobosa* in deep shade of a subtropical rain-forest, where the mean duration of leaves of the suppressed palm reached 17 years, while that of the dominant in full daylight was about three years[59]).

Moreover, also in these tree systems the endless growth in C will bring about, despite the conversion of L to D, the immense increase in R, because respiration of a certain, though very low, intensity continues in heart wood as measured by Møller[60]) in beech and spruce. The R will eventually approach and exceed the total amount of P_s, as the latter is limited by F and impinging light intensity. This will cause the overmatured tree to cease its growth, together with the decrease of F which induces the "reduced" dry-matter reproduction.

It should be emphasized here that, as these five types of reproduction systems are only an abstraction from complicated movements of matter in plant life, more comprehensive investigations should be undertaken with combining these representative reproduction systems to discover the real movement of matter in the plant and plant community and to collect quantitative data especially concerning the interrelationships between leaf, stem and root in their functions and the relation of respiration to photosynthetic activity and to growth of the plant. In these investigations the dry-matter reproduction in a shorter term naturally comes to the front. The velocity and period of the reproduction and transformation of product — turn-over — are the highly important factors in dry-matter reproduction as a whole, and these are decided by physiological factors, such as respiration, photosynthesis, growth hormones, and by environmental factors, such as temperature, water[43]) and nutrient

salts[3,4,46,47]). Further analyses and syntheses not only at individual level but also at community level of these subjects are of real necessities more fundamentally to elucidate ecological and sociological problems. Some aspects as to the relationship between the growing velocity and turn-over of dry matter in the plant will be treated in another paper of this series.

An instance of application — Shade tolerance of *Pinus* and *Picea*

It may be worth while to cite an instance of application of these reproduction systems to shade tolerance of *Pinus silvestris* and *Picea excelsa*, whose photosynthetic curves were determined by Stålfelt[40]). These two species, pine and spruce, are generally distinguished respectively as a typical sun- and a shade-tree. His experiments, however, have revealed that the compensation point in the pine shade-needle was 1.4 per cent but that in the spruce one, 2.8 per cent, and the photosynthesis in the former was higher than that in the spruce over the whole range of illumination. The photosynthesis values in Table 2, which were used for the calculation of the dry-matter reproduction, were obtained from Stålfelt's data[40]) with the following assumptions that photosynthetic hours were 12 hrs. × 180 days, the conversion factor between CO_2 and plant body was 0.65, and dry-matter percentage in both species, 35 per cent. Respiration rates of 0.3 and 0.1mg. CO_2/g. fresh weight/hr. measured respectively in the pine and spruce needles corresponded to 1.8 and 1.2mg. dry-matter/mg. dry matter/ann.; 90 days in the vegetation periods being assumed of high temperatures and 90 days, of low temeperatures. Other characteristics in the table were decided by collating several concerned studies[6,10,25,28,29,33,40,56,61–63]). The survival rate (v_l) of living tissue in C was assumed as 0.5 in both species.

Table 2. Characteristics of *Pinus silvestris* and *Picea excelsa* used for the calculation of growth curves in Fig. 1 of the seedlings. Values are determined on the basis of the data of Stålfelt[40]) and others.

	Seed dry weight	Seedling dry weight	Trans-formation factor	Distri-bution ratio $\Delta C/\Delta F$	Longevity of leaves	Survival rate v_l	Respiration (mg. dry wt./mg. dry wt./ann.)	
							Leaves	Stem and root
Pinus silvestris	6.67mg.	4.00mg.	0.6	0.5/0.5	2 yrs.	0.5	1.8	1.0
Picea excelsa	6.67mg.	4.00mg.	0.6	0.6/0.4	5 yrs.	0.5	1.2	0.3

	Photosynthesis of leaves[1]) (mg. dry wt./mg. dry wt./ann.)												Physiological compensation point of shade-leaves
Rel. light intensity	100%	20%	15%	13%	12%	11%	10%	9%	8%	7%	6%	5%	
Pinus silvestris	11.6*	6.2	5.3	4.8	4.6	4.4	4.1	3.9	3.6	3.3	3.0	2.7	1.4%
Picea excelsa	6.9*	4.6	3.8	3.3	3.1	2.9	2.7	2.4	2.2	2.0	1.7	1.4	2.8%

1) The values marked with sign * are determined for the sun-leaves, and the others, for the shade-leaves.

The repeated calculation by substituting these values into a reproduction schema

(the perennial-leaf tree type) gives rise to a growth curve of each tree species under a given light condition. Naturally the stronger the illumination, the faster is the growth of both species, and in a certain weak illumination the young tree will be killed by means of the deficiency of matter production (Fig. 1), or more directly by reduction of the photosynthetic system (Fig. 2). In *Pinus silvestris* the survival of the seedling can be ensured at or above a light intensity of 12 per cent, but the seedling of *Picea excelsa* can continuously grow even at a light intensity of 9 per cent, despite that the latter possesses rather disadvantageous characters for shade tolerance, i. e. higher C/F ratio, lower photosynthetic capacity and higher compensation point. These results indicate clearly that the shade tolerance is not merely decided by the photosynthetic characters of leaves, but also largely by the longevity of leaves (2 years in *Pinus*, 5 years in *Picea*) and the respiration rate in non-photosynthetic system. When $F(a-r)=L\cdot r_l$, no net production occurs. The amount of F of a plant is mainly decided by the amount and distribution ratio of dry-matter produced by photosynthesis and

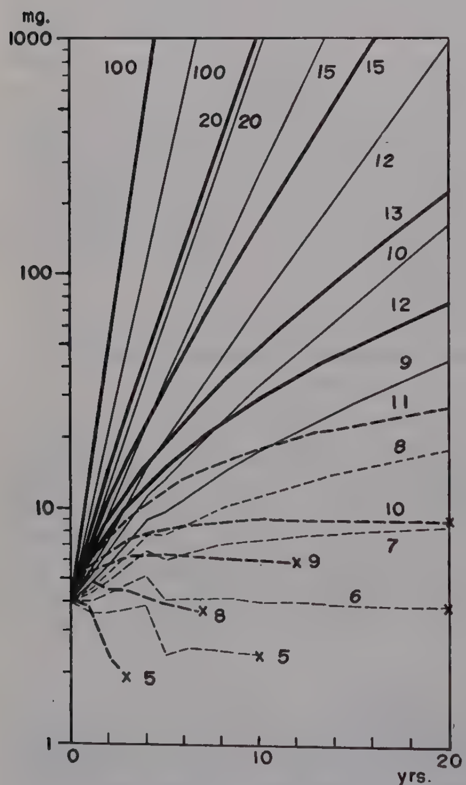


Fig. 1.

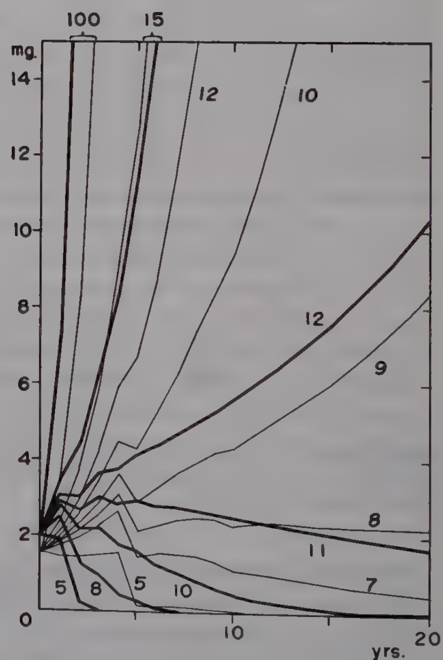


Fig. 2.

Fig. 1. Growth curves in dry weight of *Pinus silvestris* (thick lines) and *Picea excelsa* (thin lines) at various light intensities, calculated from the data of Stålfelt[40]) and others (see the text and Table 2). Solid lines: growing seedlings. Broken lines: dying seedlings. Numerals at the curves indicate relative light intensities.

Fig. 2. Growth in dry weight of the photosynthetic system of *Pinus silvestris* (thick lines) and *Picea excelsa* (thin lines), whose growth curves in total dry weight have been illustrated in Fig. 1. Numerals at the curves indicate relative light intensities.

the longevity of leaves; for shade tolerance a relative increase in leaf area against the plant weight under low light condition is also one of the cardinal factors in broad-leaved plants[32]). The turn-over can hardly play any important role in the shade tolerance because of low productivity of leaves under the condition concerned. Above a light intensity of 20 per cent the pine can grow faster than the spruce, and the growth of both seedlings continues almost exponentially in a strong illumination (see Fig. 1).

Summary

1. For elucidation of phytoecologial and -sociological problems the key function is the dry-matter production in plants or plant communities, which is maintained only through the dry-matter reproduction. The essential processes in the latter are matter production by photosynthetic system and transformation of the product into new production system. The increase of photosynthetic system is the indispensable factor for expanded reproduction in the vigorously developing plant.

2. The characteristics of reproduction systems are discussed mainly in relation to longevity of leaves, formation of seeds or tubers, enlargement of non-photosynthetic system. Accordingly five schemata were presented for typical dry-matter reproduction systems, i.e. annual herb, perennial herb, permanent herb, annual-leaf tree and perennial-leaf tree systems (cf. Table 1).

3. The shade tolerance of *Pinus silvestris* and *Picea excelsa* was elucidated in the light of dry-matter reproduction, on the basis of the data of Stålfelt and others. It was clearly demonstrated thereby that the longevity of leaves and the low respiration in non-photosynthetic system are cardinal factors for shade tolerance, besides the characters of leaves in photosynthesis.

References

1) Boysen Jensen, P., Die Stoffproduktion der Pflanzen, Jena (1932). 2) Monsi, M., and Saeki, T., Jap. J. Bot. **14**: 22 (1953). 3) Müller, D., Planta, **16**: 1 (1943). 4) ——, and Larsen, P., ibid. **23**: 501 (1935). 5) Larsen, P., ibid. **32**: 341 (1941). 6) Møller, C.M., Det forst. Forsøgsvaesen **17**: 1 (1944). 7) ——, J. Forest. **45**: 393 (1947). 8) Boysen Jensen, P., Det Kgl. Danske Videnskabernes Selskab. Biol. Med. **21**: No. 2 (1949). 9) ——, ibid. **21**: No. 3 (1949). 10) Møller, C.M., Müller, D., and Nielsen, J., Det forst. Forsøgsvaesen, **21**: 253,273,327 (1954). 11) Kasanaga, H., and Monsi, M., Jap. J. Bot. **14**: 304 (1954). 12) Hogetsu, K., and Ichimura, S., ibid. **14**: 280 (1954). 13) Monsi, M., and Oshima, Y., ibid. **15**: 60 (1955). 14) Satoo, T., Nakamura, K., and Senda, M., Bull. Tokyo Univ. Forests, **48**: 65 (1955). 15) ——, Kunugi, R., and Kumekawa, A., ibid. **52**: 33 (1956). 16) ——, and Senda, M., ibid. **54**: 71 (1953). 17) Ichimura, S., Bot. Mag. Tokyo, **68**: 7 (1956). 18) ——, ibid. **68**: 219 (1956). 19) Kusumoto, T., Jap. J. Ecol. **7**: 126 (1957). 20) Takeda, T., and Kumura, A., Proc. Crop. Sci. Soc. Jap. **26**: 165 (1957) 21) Saeki, T. and Nomoto, N., Bot. Mag. Tokyo, **71**: 235 (1958). 22) Iwaki, H., Jap. J. Bot. **16**: 210 (1958). 23) ——, ibid. **17**: 120 (1959). 24) Saeki, T., and Kuroiwa, S., Bot. Mag. Tokyo, **72**: 27 (1959). 25) Midorikawa, B., Ecol. Rev., **15**: 83 (1959). 26) Tazaki, T., Bot. Mag. Tokyo, **72**: 247 (1959). 27) Hogetsu, K., Oshima, Y., Midorikawa, B., Sakamoto, M., Tezuka, Y., Mototani, I., and Kimura, M., Jap. J. Bot. **17** (in press). 28) Polster, H., Die physiol. Grundl. d. Stofferzeug. im Walde, München (1950). 29) Pisek, A., and Winkler, E., Planta, **51**: 518 (1958). 30) Watson, D.J., Ann. Bot. N.S. **11**: 375 (1947). 31) ——, Advanc. Agron. **4**; 101 (1952). 32) Blackman, G.E., and Wilson, G.L., Ann. Bot. N.S. **15**: 373 (1955). 33) Ovington, J.D., ibid. **21**: (1957). 34) Watson, D.J., ibid. **22**: 37 (1958). 35) Clarke. G.L., Elements of ecol. New York (1954). 36) Odum, E.P., Fundamentals of ecol. 2ed. Philadelphia (1959). 37) Boysen

Jensen, P., Die Elemente d. Pflanzenphysiol. Jena, 277 (1939). 38) ——, Plante Fysiologie, Copenhagen, 260 (1946). 39) Baker, F.S., Principles of Silvicult. New York, 291 (1950). 40) Stålfelt, M.G., Medellanden från Statens Skögsforsöksanstalt, **18**: 221 (1921). 41) Davidson, J.L., and Philip, J.R., Climatol. and Microclimatol. UNESCO. 181 (1958). 42) Saeki, T., Bot. Mag. Tokyo. **73**: 55 (1960). 43) Totsuka, T., and Monsi, M., ibid. **72**: 367 (1959). 44) Tazaki, Y., ibid, **72**: 68 (1959). 45) Saeki, T., ibid, **72**: 404 (1959). 46) Tezuka, Y., ibid, **71**: 181 (1958). 47) ——, ibid. **72**: 101 (1959). 48) Braun-Blanquet, J., Pflanzensoziologie, 2 Aufl. Wien, 79 (1951). 49) Raunkiaer, C., The life froms of plants, Oxford (1934). 50) Kira, T., Ogawa, H., and Sakazaki, N., J. Inst. Polytech. Osaka City Univ. **4**: 1 (1953). 51) Kuroiwa, S., Bot. Mag. Tokyo, **73** (in press). 52) ——, ibid. **73** (in press). 53) Warming, E., Oecology of plants, Oxford, 356 (1909). 54) Nomoto, N., Kasanaga, H., and Monsi, M., Bot. Mag. Tokyo, **72**: 450 (1959). 55) Molisch, H., Die Lebensdauer d. Pflanze, Jena (1929). 56) Stålfelt, M.G., Meddelanden från Statens Skogsförsöksanstalt, **21**: 181 (1924). 57) Hiramatu, K., Ecol. Rev. **5** 25 (1939). 58) Kuroiwa, S., Bot. Mag. Tokyo, **73** (in press). 59) Oka, G., Rept. Natural Monument, Bot. **16**: 51 (1936). 60) Møller, C.M., and Müller, D., Det forst. Forsøgsvaesen, **15**; 113 (1938). 61) Bühler, A., Der Waldbau, Stuttgardt, Bd. 2. 383 (1922). 62) Harada, Y., Rept. Hokkaido Forest Exp. Stat. Imp. Forestry Agency **1**: 1 (1942). 63) Tazaki, T., Bull. Physiograph. Sci. Res. Inst. Univ. Tokyo. No. 7, 20 (1951).

摘　　要

植物における物質再生産 1.
物質再生産模式

門　司　正　三

植物の物質生産はその生態学的，また社会学的生活の基礎をなすが，それは物質再生産によってはじめて維持される．再生産過程は光合成系による物質生産過程と，その生産物が再び新しい生産系に転形される過程とにわかれる．一般に植物が生長を増加させるには光合成系の増大による拡大再生産が必要である．

葉の寿命，種子あるいは塊茎・地下茎などの形成，非光合成系の増大，およびその呼吸などについて考察して，草本系（一年生，多年生，永年生）および木本系（一年生葉，多年生葉）の5基本模式を得た(表1)．この模式に，Stålfelt の光合成曲線などを適用して，マツとトウヒの生長曲線を作成し(図1,2)，葉の光合成特性のほかに，葉の寿命，非光合成系の呼吸などが，耐陰性に強く影響する要因であることを明らかにした．（東京大学理学部植物学教室)

Studies on the Light Controlling Flower Initiation of *Pharbitis Nil*. V. On the Light Following the Inductive Dark Period

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

Received August 18, 1959

It has been known that the light intensity following, as well as preceding, an inductive dark period has a pronounced effect on initiation and development of inflorescences in *Xanthium pennsylvanicum*, and that the flowering response is increased by high-intensity light[1]. Recently it has been reported by Carr that the depressing effect of the absence of high-intensity light following a dark period in *Xanthium* may be completely overcome by supplying sucrose to the leaf[2]. Furthermore, it is well known in *Xanthium* that a brief light period interpolated between an inductive dark period and a further period of darkness is inhibitory to flowering, the degree of inhibition depending on the length of the second dark period[3].

Thus, the intensity of the illumination following an inductive dark period has an important effect on photoperiodic induction of flowering in a short-day plant.

The present investigation was performed to examine the effect of intensity and quality of light in the illumination period following an inductive dark period on flower initiation of *Pharbitis* seedlings.

As has been reported previously[4,5], illumination of far-red light preceding the inductive dark period inhibits flower initiation of *Pharbitis* strongly, and the flower inhibitory effect of low intensity light irradiation preceding an inductive dark period is obvious only when far-red light is included in it. Therefore, the effects on flower initiation of strong far-red light and low intensity light with or without far-red light given following an inductive dark period were investigated.

Material and Methods

The material and the procedure of experimentation were similar to those described in a previous paper[4].

Red light (600–700 mμ) was secured from a pink fluorescent lamp filtered with 2 layers of red cellophane and far-red light (700–1000 mμ) from an incandescent lamp filtered with 5 cm. water, 2 layers of red and 2 layers of blue cellophane.

Experimental Results

Experiment 1. As had been reported in previous papers[4,5], illumination of far-red light of 60 kiloerg/cm.^{2}/sec. strongly inhibits flower initiation when it precedes an inductive dark period. In this experiment the effect of far-red light illumination following an inductive dark period was investigated.

The plants were placed 16 or 12 hours in darkness and subsequently exposed to far-red light of 60 kiloerg/cm.^{2}/sec. (FR) for various periods ranging from 30 minutes to 8 hours, and thereafter transferred to continuous illumination of natural daylight supplemented with incandescent light at night.

The flowering responses of the plants are shown in Table 1. Far-red light illumina-

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

Table 1. Effect of far-red light illumination following 16- and 12-hour dark periods on the flower initiation of *Pharbitis* seedlings.
Intensity of far-red light (FR): 60 kiloerg/cm.^{2}/sec.
(Treated on May 13 and dissected on May 27, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16^hd	40	100	4.1	95.0
16^hd→ 30′FR	39	100	3.7	79.5
〃 → 2^hFR	39	100	3.7	77.0
〃 → 4^hFR	38	100	3.2	55.3
〃 → 8^hFR	39	100	3.2	56.5
12^hd	40	2.5	0.0	0
12^hd→ 30′FR	39	0	0	0
〃 → 2^hFR	39	15.8	0.2	0
〃 → 4^hFR	38	10.5	0.1	0
〃 → 8^hFR	40	25.0	0.3	0

16^hd: 16-hour dark period.
16^hd→30′FR: 16-hour dark period followed by far-red light illumination of 30 minutes.
These abbreviations will be used hereafter.

tion following a 16- or 12-hour dark period has a little influence upon the flower initiation.

The far-red light illumination following 16 hours of darkness appeared to inhibit flower initiation slightly, but following a 12-hour darkness it promoted flower initiation to some extent. In several repetitions of the same experiments, similar results were obtained and the effect of far-red light is slight in all cases.

Experiment 2. Daylight fluorescent light of 10 lux (FL) including little far-red light inhibits flower initiation only a little when followed by an inductive darkness of sufficient duration (16 hours), but promotes it strikingly when followed by darkness of 12 hours or less[5,6].

In the present experiment, the effect of FL illumination following a 16- or 12-hour dark period was investigated.

Plants were exposed to the daylight fluorescent light of 10 lux for various hours following 16- and 12-hour dark periods, respectively. Control plants were exposed to high-intensity light following the 16- or 12-hour darkness. As another control, two lots of plants were exposed to the daylight fluorescent light of 10 lux for 24 and 48 hours without any dark period. Results are shown in Table 2.

All lots which were subjected to a 16-hour dark period followed by various durations of FL were induced to flower as much as the control plants with which the dark period has followed by high intensity light. On the other hand, the FL illumination following a 12-hour dark period promoted flower initiation considerably. The flower promoting effect of this FL increased with increasing duration. Control plants which were exposed to the FL for 24 and 48 hours initiated no flower bud. Thus, low intensity light illumination following an inductive dark period does not inhibit flower initiation in *Pharbitis* seedlings, but promotes it when preceded by 12-hour darkness.

Table 2. Effect of the illumination of daylight fluorescent light of 10 lux following 16- and 12-hour dark periods on flower initiation of *Pharbitis* seedlings.
(Treated on June 7-9, dissected on June 23, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16hd	42	100	4.3	97.7
16hd→ 4hFL	37	100	4.6	100
〃 → 6hFL	39	94.9	4.3	92.3
〃 → 8hFL	40	100	4.6	95.0
〃 → 12hFL	37	100	4.2	94.6
〃 → 18hFL	36	100	4.3	100
〃 → 24hFL	37	100	4.5	100
〃 → 30hFL	34	100	4.8	100
〃 → 36hFL	39	100	4.5	100
〃 → 42hFL	35	100	4.6	100
〃 → 48hFL	29	100	4.3	86.2
12hd	38	94.8	1.7	0
12hd→ 2hFL	38	100	3.1	7.9
〃 → 4hFL	38	100	3.7	13.2
〃 → 6hFL	38	100	4.0	36.8
〃 → 8hFL	37	100	3.3	27.0
〃 → 12hFL	38	100	3.4	26.3
〃 → 18hFL	38	100	2.8	10.5
〃 → 24hFL	39	100	3.7	35.9
〃 → 30hFL	32	100	3.0	43.8
〃 → 36hFL	37	100	3.3	54.1
〃 → 42hFL	32	100	4.1	62.5
〃 → 48hFL	37	100	4.2	59.5
24hFL	38	0	0	0
48hFL	39	0	0	0

FL: Daylight fluorescent light of 10 lux.

Assuming that the last process of the inductive dark period can proceed under FL to some extent, the results can be interpreted plausibly. That is: a 16-hour dark period induces maximum flowering response and further lengthening of the dark period does not increase flowering response; therefore, even if the last process of the inductive dark period proceeds under FL following 16-hour darkness, flowering response is not promoted. On the other hand, a 12-hour dark period can not induce maximum flowering response and further lengthening of the dark period increases flowering response remarkably; therefore, if the last process of the inductive dark period proceeds under FL following 12-hour darkness, flowering response is promoted.

Experiment 3. An experiment similar to the second one was carried out but the far-red light of 120 erg/cm.^{2}/sec. was mixed with FL (FL+FR). As shown in Table 3, when the low-intensity light following an inductive dark period contains far-red light, flower initiation of *Pharbitis* seedling is scarcely influenced when the dark period is

Table 3. Effect of FL+FR illumination following 16- and 12-hour dark periods on flower initiation of *Pharbitis* seedlings.

FL+FR: Daylight fluorescent light of 10 lux mixed with far-red light of 120 erg/cm.^{2}/sec.

(Treated on July 3–4 and dissected on July 16, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16^hd	39	100	4.3	97.5
16^hd→ 4^h(FL+FR)	39	100	4.4	92.3
〃 → 8^h(FL+FR)	38	100	4.2	92.1
〃 →12^h(FL+FR)	34	100	4.3	91.2
〃 →18^h(FL+FR)	36	100	4.3	94.5
〃 →24^h(FL+FR)	35	100	4.3	91.4
〃 →30^h(FL+FR)	37	100	4.4	94.7
〃 →36^h(FL+FR)	31	100	4.6	96.8
〃 →48^h(FL+FR)	34	100	4.6	88.3
12^hd	40	90.0	1.1	0
12^hd→ 4^h(FL+FR)	38	100	1.6	0
〃 → 8^h(FL+FR)	38	100	1.7	2.6
〃 →12^h(FL+FR)	38	94.8	1.5	0
〃 →18^h(FL+FR)	39	89.9	1.3	0
〃 →24^h(FL+FR)	37	89.2	1.4	0
〃 →30^h(FL+FR)	40	95.0	1.8	2.5
〃 →36^h(FL+FR)	36	97.0	1.9	5.6
〃 →48^h(FL+FR)	34	100	1.8	0
24^h(FL+FR)	40	0	0	0
48^h(FL+FR)	41	0	0	0

16 hours, but it appears to be promoted slightly when the dark period is 12 hours. Control plants which were subjected to such light for 24 and 48 hours did not initiate any flower bud.

FL illumination following a 12-hour dark period promotes flower initiation more obviously than FL+FR. The last process of the inductive dark period can be assumed to be largely prevented by the far-red light.

Experiment 4. Plants were placed in darkness for 16 or 12 hours, at the end of which they were irradiated for 5 minutes with red light of 3000 erg/cm.^{2}/sec., and immediately thereafter subjected to various hours for a second dark period. The flowering responses of these plants are shown in Table 4. The second dark period following 16 hours of darkness exerts no influence on flower initiation, but that following 12 hours of darkness promotes flowering response remarkably. The flower inhibitory effect of the second dark period which is obtained in *Xanthium*[3]) is not observed in *Pharbitis* seedlings. The last process of the inductive dark period is presumably stable to the brief red light.

Experiment 5. Plants were given a 16-hour period consisting of two parts, a certain number of hours in the dark followed by a light period of FL or FL+FR.

Table 4. Effect of a second dark period of 2–8 hours given following a 16- or 12-hour dark periods and 5 minutes of red light.
Intensity of red light: 3000 erg/cm.^{2}/sec.
(Treated on April 22, and dissected on May 13, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
$16^{h}d$	38	100	5.0	100
$16^{h}d \rightarrow 5'R \rightarrow 2^{h}d$	37	100	5.0	100
〃 → 〃 → $4^{h}d$	40	100	5.1	100
〃 → 〃 → $8^{h}d$	35	100	5.0	100
$24^{h}d$	39	100	5.2	100
$12^{h}d$	38	100	1.1	0
$12^{h}d \rightarrow 5'R \rightarrow 2^{h}d$	40	100	4.3	75.0
〃 → 〃 → $4^{h}d$	40	97.5	4.6	90.0
〃 → 〃 → $8^{h}d$	39	100	4.8	94.9
$20^{h}d$	40	100	4.9	97.5

Table 5. Flowering response of *Pharbitis* seedlings which were subjected to a 16-hour period consisting of various hours of darkness and followed by FL or FL+FR.
FL: Daylight fluoresceut light of 10 lux.
FL+FR: Daylight fluorescent light of 10 lux mixed with far-red light of 120 erg/cm.^{2}/sec.

Group	Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
I*	$16^{h}d$	37	100	4.1	100
	$14^{h}d \rightarrow 2^{h}FL$	40	100	4.1	97.5
	$12^{h}d \rightarrow 4^{h}FL$	39	100	3.4	17.9
	$10^{h}d \rightarrow 6^{h}FL$	39	12.8	0.2	0
	$8^{h}d \rightarrow 8^{h}FL$	38	2.6	0.0	0
	$14^{h}d$	41	100	4.3	100
	$12^{h}d$	39	84.6	2.1	5.1
	$10^{h}d$	40	0	0	0
	$8^{h}d$	41	0	0	0
II**	$16^{h}d$	38	100	4.8	100
	$14^{h}d \rightarrow 2^{h}(FL+FR)$	38	100	4.9	97.4
	$12^{h}d \rightarrow 4^{h}(FL+FR)$	40	100	2.2	2.5
	$10^{h}d \rightarrow 6^{h}(FL+FR)$	39	7.7	0.1	0
	$8^{h}d \rightarrow 8^{h}(FL+FR)$	38	0	0	0
	$14^{h}d$	39	100	4.3	76.9
	$12^{h}d$	39	87.2	0.9	0
	$10^{h}d$	39	0	0	0
	$8^{h}d$	39	0	0	0

* Treated on May 22 and dissected on June 6, 1958.
** Treated on May 23 and dissected on June 7, 1958.

The first group of plants was subjected to 16-, 14-, 12-, 10- and 8-hour darkness followed by 0-, 2-, 4-, 6- and 8-hour FL, respectively. The second group was treated in the same way as the first group but far-red light of 120 erg/cm.^{2}/sec. was mixed with FL (FL+FR). Control plants for both groups were placed in darkness for 14, 12, 10 and 8 hours and then in high-intensity light. Results are shown in Table 5.

In the first group, the plants placed in darkness for 16 hours and those given 14 hours of darkness followed by high-intensity light or 2-hour FL, were induced to flower to the same extent. But with the further decrease of the dark period, accompanied by an increasing duration of the FL, flowering response is reduced gradually, and only one out of 38 plants which were placed in darkness for 8 hours followed by 8-hour FL illumination initiated a flower primordium.

Comparing the flowering responses of the plants subjected to 12∼8 hours of darkness followed by 4- to 8-hour FL illumination, and those in darkness for the same length of time but followed by high-intensity light illumination, the former initiated slightly more flower buds than the latter.

From these results it appears that the flower-inducing process taking place in the last 6∼8 hours of the 16-hour dark period proceeds slightly under FL.

In the second group, similar results were obtained, but the last process of the inductive dark period appears to be inhibited more strongly by FL+FR than by FL, but less than by high-intensity light.

Discussion

Low-intensity light illumination following an inductive dark period of 16 hours does not inhibit the flowering response of *Pharbitis* seedlings whether far-red light is included or not. Furthermore, a second dark period which is given following 16 hours of darkness and 5 minute illumination with red light (3000 erg/cm.^{2}/sec.) does not inhibit flowering response.

These phenomena observed with *Pharbitis* seedlings offer opposition to the results obtained with *Xanthium* [1,2,3]), in which the flowering response was reduced strikingly by low-intensity light or a second dark period following an inductive dark period.

From the results of experiments 2 and 5, it appears that the last phase of the 16-hour dark period proceeds to some extent under daylight fluorescent light of 10 lux (FL) but that the first phase of the inductive dark period proceeds more readily under FL[5,6,7]).

As had been reported previously, the first process appears to proceed easily under far-red light[5,6,7]), but the present experiment shows that the last process is inhibited considerably by far-red light.

From the present and the previous experiments[4,5,6,7,8]), it is concluded that the inductive dark process is relatively stable to light during the first but less stable during the last phase.

Summary

1) Far-red light or low-intensity light illumination following an inductive dark period of 16 hours has no significant influence upon flower initiation of *Pharbitis* seedlings.

2) Illumination with low-intensity light containing little far-red radiation promotes flowering response to some extent if it follows a dark period of 8∼12 hours, but far-red light illumination does not.

3) The flower-promoting effect of low-intensity light illumination following a dark period of 12 hours or less decreases considerably if the far-red light of low intensity is mixed with it.

4) A second dark period separated from an inductive dark period by a brief exposure to red light does not inhibit flowering response when the first dark period is 16 hours, and promotes flowering strikingly when the first period is 12 hours.

5) The last phase of the inductive dark period appears to be relatively light stable, but less stable than the first phase.

References

1) Mann, L. K., Bot. Gaz., **102**: 339 (1940). 2) Carr, D. J., Physiol. Plant., **10**: 249 (1957). 3) Lockhart, J. A., and Bonner, J., Bot. Gaz., **116**: 133 (1954). 4) Takimoto, A., and Ikeda, K., Bot. Mag. Tokyo, **72**: 137 (1959). 5) —— and ——, ibid., **72**: 181 (1959). 6) ——, and ——, ibid., **72**: 388 (1959). 7) ——, and ——, ibid., **73** : 37 (1960). 8) ——, ——, and Imamura, S., ibid., **71**: 317 (1958).

摘 要

アサガオの花芽形成を支配する光条件について
V. 暗期後の光について

滝 本 敦・池 田 勝 彦

1) 16時間の暗期後に与えた近赤外光または弱光 (10 lux) は，アサガオの花芽形成にあまり影響を与えない．

2) 8〜12 時間後に与えた近赤外光を含まない弱光 (10 lux) は花芽形成をかなり促進する．しかし近赤外光はこの促進効果を持たない．

3) 上記弱光の花芽形成促進効果は，弱い近赤外光 (120 erg/cm.²/sec.) を同時に与えることによって妨げられる．

4) オナモミでは暗期後5分間赤色光を与え，それに続いてさらに第2の暗期 (8時間)を与えると花芽形成が抑制されるといわれているが[3]，アサガオではこのような現象は見られず，第1の暗期が 11 時間の場合には逆に花芽形成が促進される．

5) 花芽形成に有効な暗期の最後の段階は比較的光に安定で，弱光下でもある程度進み得，また短時間の赤色光照射に対しても安定だと考えられる．しかし暗期の初期段階程光に安定ではない．

（京都大学農学部 応用植物学研究室）

On the Ascorbic Acid in the Plumule of Indian Lotus Seed

by Kiyonobu TOYODA*

Received August 28, 1959

The writer has been studying about the properties of the fruit or seed of Indian lotus plant and already reported that unripe seed could germinate[1]), the components of gas contained in the cavity of the fruit did not change for ages[2]), and chlorophyll *a* and *b* were detected in the green portion of the plumule[3]).

This report deals with a reducing substance which is contained in the plumule and seems to consist of ascorbic acid for the most part. The writer is of the opinion that the ascorbic acid in the seed has something to do with a long longevity of Indian lotus seed, and intends to present the results of the ascorbic acid measurements in some angiospermous seeds.

Materials and Method

Under various conditions, fruits of Indian lotus plant (*Nelumbo nucifera* Gaertn.) were investigated. Also the seeds of some species of *Citrus*, *Fortunella*, *Pisum* and *Glycine* were employed. The details of materials used in this study are shown in Table 1.

Table 1. Materials

Species	Part
Nelumbo nucifera Gaertn.	Plumule, cotyledon, seedling, and leaf
Citrus sinensis Osbeck	Green embryo
C. depressa Hayata	〃
C. Kinokuni Hort. ex Tanaka	〃
C. Oto Hort. ex Y. Tanaka	〃
Fortunella margarita Swingle	〃
Cucurbita moschata Duch.	Green tissue of inner part of the seed
C. maxima Duch.	〃
Pisum sativum L.	Green cotyledon
Glycine Max Merrill	〃
Citrus Natsudaidai Hayata	White cotyledon
Pisum sativum L.	〃
Glycine Max Merrill	〃

Iijima *et al.*[4]) detected ascorbic acid in plant cells by means of acidified silver nitrate solution, and, comparing the results with those of the indophenol method, stated that ascorbic acid should be examined by both methods. The writer used mostly the indophenol method described by Fujita[5, 6]). According to this method, the following substances are estimated besides ascorbic acid: Ferrous salt, sulphite, SH compound, reductone and reductive acid. These substances are generally small in quantity, so that the reducing substance detected by this method might be considered to

* Fujisawa Higher School attached to Nihon University, Fujisawa, Kanagawa Pref., Japan.

be ascorbic acid for the most part. Every test employed in the present study was repeated from two to ten times and the average was taken. These experiments were carried out from December, 1958 to April, 1959.

Experimental Results

The amount of ascorbic acid (reduced form) and dehydroascorbic acid (oxidized form) found in the plumules and cotyledons of *Nelumbo* seeds are given in Table 2.

Table 2. The contents of ascorbic acid in the plumule and cotyledons of *Nelumbo* seeds (mg. %).

Plants	Reduced form	Oxidized form	Total
1. Plumule of a fruit 3 months after the maturation at a lotus field of Kamakura.	26.2	212.3	238.5
2. Plumule of the same fruit 15 months after the maturation.	25.6	207.9	233.5
3. Plumule of the same fruit preserved in a bottle covered with water and mud for 15 months.	29.0	212.6	241.6
4. Plumule of a fruit, taken out of mud of a lotus field at Fujisawa. The fruit was presumably more than several years.	29.1	214.2	243.2
5. Plumule of a fruit, taken from a receptacle found at Akanuma pond in Niigata Pref. in 1954.	24.1	214.5	238.6
6. Cotyledon of the fruit.	9.8	43.7	53.5

The change of ascorbic acid content with temperature is shown in Table 3. The fruits were put at 0°, 25°, or 50°, and ascorbic acid of the plumules was examined.

Table 3. The content of ascorbic acid in the plumule of the fruit of *Nelumbo* under the various temperatures (mg. %).

Temperature	Days	Reduced form	Oxidized form	Total
0°	7	25.8	208.4	234.2
〃	14	23.6	209.7	233.3
〃	21	22.5	207.1	229.6
25°	7	26.3	214.9	241.2
〃	14	26.7	215.3	242.0
〃	21	27.8	213.8	241.6
50°	7	27.1	214.0	241.1
〃	14	29.0	212.5	241.5
〃	21	30.8	214.4	245.2

The *Nelumbo* fruit was cut off at the base of the fruit-coat and soaked in water, and allowed to stand in the light (in the room) as well as in the dark. The contents of ascorbic acid in these seedlings are given in Table 4. After 5 days soaking in water, the seedlings sprouted 2–8 mm. in length and they obtained an average weight of 161 mg., i.e. about 5.5 times of that of the plumules 29.5 mg. After 10 days

soaking in water, the seedlings were 6–9 cm. long and 344 mg. in average weight: they were about 11.7 times of the plumule on a fresh weight basis. The average dry weight of these seedlings was about 40 mg. as compared with 26.6 mg. of the plumules.

Table 4. The content of ascorbic acid in the seedling and in the normal leaf (mg. %).

Plants	Days of soaking in water	Reduced form	Oxidized form	Total
Seedlings in the light (in the room)	5	11.3	37.8	49.1
Seedlings in the dark	〃	9.1	35.0	44.1
Seedlings in the light	10	27.1	4.4	31.5
Seedling in the dark	〃	11.5	3.3	14.8
Normal leaf (planted in the Gempei pond at Kamakura)		226.5	22.3	248.8

The ascorbic acid contents in green embryos of some rutaceous plants and in the green tissues of some cucurbitaceous seeds are also examined, and the results are given in Tables 5 and 6.

Table 5. The contents of ascorbic acid in the embryos of some rutaceous plants (mg. %).

Species	Color of cotyledon	Reduced form	Oxidized form	Total
Fortunella margarita (fresh)	Green	19.2	38.0	57.2
,, (1 month after being taken from the fruit)	〃	8.1	45.3	53.4
Citrus Kinokuni (do., 1 month)	〃	11.1	31.9	43.0
C. sinensis (do., 3 months)	〃	12.2	32.1	44.3
C. depressa (do., 5 months)	〃	9.6	35.6	45.2
C. Oto (do., 7 months)	〃	7.1	29.0	36.1
C. Natsudaidai (fresh)	White	6.2	25.6	31.8

Table 6. The contents of ascorbic acid in the green tissues of some cucurbitaceous seeds (mg. %).

Species	Reduced form	Oxidized form	Total
Cucurbita moschata (fresh, the green tissue retained about 90% moisture)	10.2	10.1	20.3
C. maxima (5 months after being taken from the fruit, retained 10% moisture)	7.5	19.0	26.5

Furthermore the seeds of soy bean and pea, green as well as white, were measured in ascorbic acid content. The results are shown in Table 7.

Table 7. The contents of ascorbic acid in the cotyledons of leguminous plants (mg. %).

Species		Color of cotyledon	Reduced form	Oxidized form	Total
Pisum sativum (fresh)		Green	15.7	36.4	52.1
〃	(after 10 months)	〃	8.7	34.8	43.5
Glycine Max (1 month being taken from the fruit)		〃	13.2	41.9	55.1
〃	(after 5 months)	Yellowish white	8.5	14.6	19.1
Pisum sativum (fresh)		White	15.1	23.6	38.7
〃	(after 10 months)	〃	8.3	26.3	34.6

Discussion

All reducing substances in plant tissues are not necessarily ascorbic acid, but those in green tissues are to be regarded as ascorbic acid. Fujita *et al.*[7]) reported that reducing value represents the true value of ascorbic acid estimated by the enzyme method in the leaves having chlorophylls such as soft greens (*Brassica Rapa* var. *lacinifolia*) and turnip (*Brassica Rapa* var. *glabra*). Even in seeds having no chlorophylls such as French beans (*Phaseolus vulgaris*) and Indian corn (*Zea Mays*), total reducing value coincides also with the true value of ascorbic acid. Only in wheat and chestnut, all reducing substances were not ascorbic acid.

In the green plumule of *Nelumbo* seed, the reducing substances detected by the indophenol method were considered to be ascorbic acid for the most part, judging from the results mentioned above. A little ascorbic acid and much of dehydroascorbic acid were found in the plumule of *Nelumbo* seed. In old fruits, the amount of ascorbic acid remained almost unchanged, and it was smaller in the fruits placed in the air than in those placed in water as well as taken from the mud of lotus field.

The ascorbic acid was slightly affected by temperature, and increased at 25° and 50° and decreased at 0° after 7-day treatment. These facts agree with Sugawara's result[8]) that the ascorbic acid in soy been and wheat seed increased with higher temperatures (30°, 35°). In a previous paper[2]), the writer reported the amount of carbon dioxide contained in *Nelumbo* fruit increased with a high temperature (60°). This fact seems to correlate with Sugawara's result[9]) that ascorbic acid in the leaves is almost directly proportional to the carbon dioxide concentration of the air. Franke[10]) also observed that the increase in the respiration of seedlings of *Sinapis alba* and of slices of potato tissue ran parallel with an increase in ascorbic acid of the tissue.

In seedlings 5 days after germination, the amount of ascorbic acid decreased, but the amount of ascorbic acid per one seedling increased evidently and dehydroascorbic acid was almost unchanged, while the seedling had grown up to 5.5 times of the plumule. Ascorbic acid and dehydroascorbic acid were found in larger quantity in the seedling which germinated in the light than the one grown in the dark. In the 10-day seedlings, which have grown to 11.7 times of the plumule, the amount of ascorbic acid per one seedling increased, but dehydroascorbic acid decreased extremely, and the amount of ascorbic acid was far larger in the light than in the dark. These facts correspond to Sugawara's result[8]) that the amount of ascorbic acid in the illuminated seedling after 125 hours was about 65% greater than in the etiolated seedling which germinated in the dark. The average dry weight of these

seedlings are about 1.5 times of that of the plumule, because the plumule has absorbed materials from cotyledons. So the amount of ascorbic acid of a seedling which germinated in the light and that of a plumule were on a dry weight basis almost unchanged (see Table 4). Foliage leaf of *Nelumbo* planted in the pond contains a considerable amount of ascorbic acid in reduced form, the total amount of ascorbic acid being almost equal to that of the plumule.

Ascorbic acid was also found in embryos of rutaceous plants, in green tissues of seeds of cucurbitaceous plants and in the cotyledons of leguminous plants. The amount of ascorbic acid in these materials, though it varies with the kinds of species, decreased with the lapse of time in general. This fact agrees with Hoover's report[11]) that the amount of ascorbic acid in Southern pea decreased slowly with the stage of maturing and the period of storage. In the seeds of cucurbitaceous plants, a similar fact is observed on a dry weight basis. There is found more ascorbic acid in green tissues than in tissues having no green pigment. It may be proved by the description of Mapson[12]) as to the findings of Giroud *et al.* and Mirimanoff that the reduced silver often appears localized within the chloroplast when the plant tissues are treated with silver nitrate.

About ascorbic acid (vitamin C) in plant tissues, many works have been reported, but most of them dealt with edible parts. Moldtman[13]) studied ascorbic acid in plant tissues physiologically and noticed the amount of ascorbic acid grew up to a maximum from noon toward afternoon. Sugawara[9,14]) measured the amount of ascorbic acid contained in the leaves of vegetable and found a similar fact. By these facts, he noticed the formation of ascorbic acid and photosynthetic activity stood in a close interrelationship. Yoshimura[15]) reported that the amount of ascorbic acid in higher plants diurnally reached a maximum in the afternoon. Regarding the seed, ascorbic acid is not found or is scanty in quantity and comparatively few works[8,11,16-21]) have been reported.

The amount of ascorbic acid in rice seed decreased with the length of storage period as Sugawara[8]) showed, and the seed which had been preserved for 5 years contained almost no ascorbic acid and a similar result was also obtained with maize seeds. However, the amount of ascorbic acid in the plumule of *Nelumbo* seed seems almost unchanged for ages. Fruits of *Nelumbo* have been supposed to have a long longevity of two thousand years, and in this period metabolism goes on extremely slowly in the fruits, so that ascorbic acid there might have some relation with this fact.

The writer wishes to express his sincere thanks to Professor S. Hattori of the University of Tokyo for his kind guidance and invaluable advice. Further he thanks cordially to Professor S. Nagami of the Yokohama University, Dr. H. Iwao of the National Institute of Nutrition and Dr. T. Sugawara of the University of Tokyo.

Summary

1. The amount of ascorbic acid (reduced and oxidized forms) contained in the plumule of *Nelumbo nucifera* and in the seeds of several other plants was measured by the indophenol method.

2. The content of ascorbic acid in the plumule of *Nelumbo* is considerably high and exists in oxidized form for the most part. This amount hardly changes in the fruit for several years.

3. There is only a little effect of temperature on the amount of ascorbic acid in the plumule of *Nelumbo*, which increases slowly at 25° and 50°, and decreases

slightly at 0°.

4. By germination, the amount of ascorbic acid increases, dehydroascorbic acid considerably decreases per one seedling, and the seedling which germinated in the light contains larger amount of ascorbic acid than that grown in the dark. Foliage leaf of *Nelubmo* contains a considerable amount of ascorbic acid, which consists of reduced form for the most part.

5. The contents of ascorbic acid in the embryos of *Citrus*, *Fortunella*, green tissues of seeds of *Cucurbita*, and cotyledons of *Pisum* and *Glycine* were measured. In these seeds, dehydroascorbic acid is also comparatively rich and the amount of ascorbic acid varies with the kinds of species and generally was found to be less in the older seed than in the new. The amount of ascorbic acid is more in the tissue having chlorophylls than in the one having no green pigment.

6. It is noticeable that a considerable amount of ascorbic acid is contained in the plumule of *Nelumbo* seed and the amount of this substance does not change for years. It seems likely to be concerned with a long longevity of *Nelumbo* fruit.

References

1) Toyoda, K., Journ. Jap. Bot., **33**: 85 (1958). 2) ——, Bot., Mag. Tokyo, **71**: 371 (1958). 3) ——, Bot. Mag. Tokyo, **72**: 159 (1959). 4) Iijima, M. *et al.*, Bot. Mag. Tokyo, **63**: 278 (1950). 5) Fujita, A., Medicine and Biology, **2**: 265 (1942). 6) ——, Medicine and Biology, **2**: 272 (1942). 7) ——, and Iinuma, S., Medicine and Biology, **6**: 211 (1944). 8) Sugawara, T., Jap. J. Botany, **14**: 125 (1954). 9) ——, Jap. Journ. Bot., **11** 343 (1941). 10) Franke, W., Planta, **44**: 437 (1954). 11) Hoover, M.W., Food Res., **20**: 469 (1955). 12) Mapson, L.W., Ann. Rev. Plant Physiol., **9**: 119 (1959). 13) Moldtman, H.G., Planta, **30**: 297 (1939). 14) Sugawara, T., Jap. Journ. Bot., **11**: 141 (1941). 15) Yoshimura, F., Bot. Mag. Tokyo, **67**: 97 (1954). 16) Matsuaka, T., Mem. College Agr. Kyoto Im. Uni., **9**: 15 (1930). 17) ——, Mem. College Agr. Kyoto Im. Uni., **9**: 23 (1930). 18) Sreenivansan, A. *et al.*, Nature, **165**: 765 (1950). 19) Miller, E.V., *et al.*, Food Res., **14**: 492 (1949). 20) Fujita, A. *et al.*, Medicine and Biology, **5**: 501 (1944). 21) ——, Medicine & Biology, **5**: 645 (1944).

摘　　要

ハスの幼芽のアスコルビン酸について

豊　田　清　修

1. ハスの果実内の幼芽および他の数種の植物の種子内に含まれるアスコルビン酸(還元型と酸化型)の量をインドフェノール法によって測定した.

2. ハスの幼芽に含まれるアスコルビン酸の量はかなり多い，そして多くは酸化型で存在する. その量は数年たった古い果実でもほとんど変らない.

3. ハスの幼芽に含まれるアスコルビン酸の量は温度によりわずかの影響を受け，25°, 50° では少し増加し，0° ではやや減少する.

4. 果実が発芽すると，幼芽1個あたりのアスコルビン酸は増加し，酸化型アスコルビン酸はかなり減少する. また明所で発芽した芽生えは暗所で発芽した芽生えより多くのアスコルビン酸を含む. ハスの普通の葉はかなりの量のアスコルビン酸を含み，その多くは還元型である.

5. ミカン属，キンカンの胚，カボチャ属の種皮の緑色の組織，エンドウ，ダイズの子葉に含まれるアスコルビン酸を測定した. これらの種子においても酸化型アスコルビン酸の量は比較的多い，アスコルビン酸の量は種類によっても違うが，一般に古い種子では新らしい種子より少ない. また緑色の組織では非緑色の組織よりアスコルビン酸の量は多い.

6. ハスの果実内の幼芽にかなり多くの量のアスコルビン酸を含んでおり，この物質の量が永年の間ほとんど変化しないことは注目すべきことである. それはハスの果実が長寿を保つことと関係がありそうである. (日本大学藤沢高等学校)

Inner Stage of Action and Eso-ecology of Plants

by Riichiro KOKETSU*

Received November 27, 1959

Introduction

Since the author origially used the so-called 'powder method'[1]) for the purpose of indicating the matter contents in plant-bodies, many works proving the effect of this method and also of other related methods have been published. While in connection with those works the concept of the inner stage of physiological activities[2]) was developed, an idea pertaining to the inner stage of action was accordingly introduced in plant physiology[3]), and several studies in regard to this concept have appeared[4,5]).

The inner stage implies the entire inner environmental factors or the inner habitat of plants, and affects any physiological function taking place on this stage. The study of the interrelation of the inner stage of action and the course of a physiological function, therefore, may be a good key to disclose the essential mechanism of a physiological function in plants. This study actually tends to be ecological rather than physiological. The inner ecology (eso-ecology or esecology), therefore, was introduced as a new term in botany[6]).

The concept of the inner stage of action and its application

The inner stage of action is the place or habitat where a physiological function takes place in the plant body. All physiological functions operate within the body of organism, although the course of a function is usually affected by the external environmental conditions. The influence of an external environmental factor on a physiologial function, however, is not direct. Its influence acts primarily on conditions in the inner stage, and secondarily, on the physiological function. It is difficult to study the exact relationship between a physiological activity and its environmental condition, even when the external environmental factors or the environmental factors in the microhabitat outside of the body are studied. For this reason the environmental factors within the body directly related to the physiological activities must be investigated.

The inner stage of action or the inner habitat includes the anatomical or histological structure and the physico-chemical property in the body[2]). The natural course of any physiological activity of the inner stage should be influenced from these two aspects. From the view point that the function and structure of the body are interrelated as the cause and effect, it may be said that the role of the so-called physiological anatomy of plants is essential. On the other hand, the physico-chemical properties of the cell and tissue have been studied extensively to clarify the cause or mechanism of a physiologial phenomenon. According to the author's concept, these methods of study are actually ecological.

Fundamental is the problem of whether it is possible to estimate effectively the matter content or the degree of function, although the degree of such properties as body temperature, sap-concentration, electrical ability, etc., can be studied with little difficulty. Since the usual percentage of matter contents is only a relative indication,

* Emeritus Prof. Kyushu Univ., Fukuoka, Japan.

it may be, at times, too obscure to determine the content value in the stage of action. In order to determine the actual matter contents in the tissues, it is necessary to estimate the amount of the matter contained in the actual volume of the body-tissues or the relative amount compared to other substances which are contained in the tissue without appreciable variation. No practical method of indication corresponding to such an idea, however, is known as yet. It was, therefore, necessary to apply methods which rendered comparatively effective results for this purpose[5]).

The 'powder method'[1,7,8]) which estimates the amounts of matter contents per unit volume of the dried tissues powder was proved to be a useful method by a series of related studies. The determination of the specific weight of tissue powder[9]), the determination of the hygroscopic ability of tissue powder[10]), and the refractometric determination of the concentration of the extract per definite volume of the dried tissue powder[11, 12]) have been attempted in many works according to the author's concept. After this concept was introduced, many useful results of physiological studies which actually should be placed in the category of ecological studies have appeared.

Meaning and role of eso-ecology

Eso-ecology is a special field of science which deals with the relationship between the physiological functions and the environmental conditions in the inner stage of action. A physiological function in the living body is not independent of other co-operating functions. In other words, it is a part of the entire life phenomena. All physiological functions are related to one another forming a functions-complex on the inner stage of action or the inner habitat of the body. It may, therefore, be a key to reveal the secret of life, to study the interrelation ecologically, which is the fundamental role of eso-ecology.

The current ecology, not only synecology but also autecology, deals with the external habitat-factors. It is a study of the conditions in the external environment or the study of the ecological conditions of the immediate space surrounding the plant notwithstanding the internal habitat factors. In physiology, the influence of the external environmental factors on the physiological functions is generally studied. The influence of any external environmental condition on a physiological function primarily effects the internal conditions which are actually directly related to the function; the physiological function is related to the body temperature, but only indirectly to the temperature of air or soil. Thus, the initial problem is to measure the body temperature for the purpose of studying the influence of temperature. To study the relation between the physiological functions and the environmental conditions, it is necessary to consider the internal conditions (the conditions of the inner stage of action). It is desirable to study a physiological function under controlled conditions. Any physiological function, however, is taking place in the inner stage of action under the influence of the complex physico-chemical and biotic factors which are the components of the stage itself. The physiological functions on this stage are influenced by one another as a biotic environmental factor. Therefore, it might be said that the eso-ecological study is essential in explaining a physiological function.

The interrelation between the assimilation and the respiration of plants is still obscure in physiology. Many advanced works, however, in attempt to solve this problem have appeared[13]); works being done from the ecological stand point. This suggests the necessity of the eso-ecological concept in physiological studies. This field in science will be systemized after further investigation, although little is known of

the basic elements constructing eso-ecology as yet.

Conclusion

The outline of the concept of the inner stage of action and eso-ecology has been proposed and described. The problems mentioned in this note should be applied to the animal and human life as well as to the plant life. Since the health of living beings depends upon the inner stage of action, and all natural or artificial damages of the inner stage result in unhealthiness, it may be worthwhile to introduce this concept to such applied sciences, as agriculture, medicine, etc. Indeed, a tendency to treat physiological works as the problems of the habitat within the body is growing rapidly.

If the final aim of physiology is to clarify physico-chemically the true nature of life, it may be of little significance to study each function separately. The eso-ecological study of the functions-complex in the living body seems to be more appropriate.

References

1) Koketsu, R., Jour. Dept. Agr. Kyushu Imp. Univ, **1**: 151 (1924). 2) Koketsu, R., Agr. and Hort. **15**: 1069, 1283 (1940) (in Japanese). 3) Koketsu, R., Agr. and Hort. **16**: 639, 823, 1017 (1941) (in Japanese). 4) Koketsu, R., Agr. and Hort. **18**: 1039, 1139 (1943) (in Japanese). 5) Koketsu, R., Agr. and Hort. **19**: 285, 391, 481, 569 (1944) (in Japanese). 6) Koketsu, R., Agr. and Hort. **23**: 1475 (1958) (in Japanese). 7) Koketsu, R., Rep. Jap. Sci. Assoc. **6**: 460 (1930) (in Japanese) 8) Koketsu, R., Hydrophysiol. of Plants 15 (1940) (in Japanese). 9) Koketsu, R., and Fukaki, S., Bult. Sci. Fakul. Terk. Kyushu Imp. Univ. **2**: 273 (1927) (in Japanese). 10) Koketsu, R., Jour. Dept. Agr. Kyushu Imp. Univ. **3**: 149 (1932). 11) Koketsu, R., Ecol. Rev. **8**: 69 (1942) (in Japanese). 12) Koketsu, R., Taguchi, Ry., and Omura, R., Bult. Sci. Fakul. Terk. Kyushu Imp. Univ. **10**: 383 (1943) (in Japanese). 13) Decker, J. P., Plant Physiol. **30**: 82 (1958).

摘　要

植物の体内舞台と体内生態学

纐　纈　理　一　郎

植物体内で行なわれる生理作用と環境要因との関係の攻究に当って必要な体内環境要因とその在り方の合理合目的な表示への関心に立脚したいわゆる組織粉末法の効果的利用から出発して，生理作用の行なわれる場としての体内舞台の概念を構成し，この舞台上で行なわれる生理作用は，舞台の構造的要因，理化学的要因および生物的環境要因としての同時に行なわれている他の生理作用などによる綜合的影響の下にあるのだから，生理作用の自然における現実の現われの追究には，作用と体内舞台との関係を生態学的に取扱う態度が必要であり，したがってここに体内生態学の成立分野があることを説いた．

なおこの概念とその運用は植物に限らず，動物ないし人間の場合にも適用され得るはずである．

（九州大学名誉教授）

Observations on the Embryo Sac Containing Double Egg Apparatus in *Triticum aestivum* L.

by Kiyochika HOSHIKAWA*

Received September 7, 1959

Introduction

During the course of embryological observations on wheat, the author met with several embryo sacs possessing double egg apparatus. As this unusual duplication of egg cells may contribute to explain the problem of the formation of twin germ grain, the author wants to describe it briefly.

In this paper, the morphological description of such embryo sacs has been reported, and further, its origin and the possibility giving rise to polyembryony have been discussed.

Materials and Methods

Triticum aestivum L. varieties, *Saitama-No. 27*, *Nōrin-No. 26* and *Nōrin-No. 42* were used as the materials. About 1,000 ovaries, collected just at their flowering times, were fixed with formalin acetic alcohol solution. The preparations of 10-15μ thick longitudinal and cross sections were made according to the simplified paraffin method designed by the author[1]). According to this method the dehydrated samples are soaked in crystal violet-containing paraffin for about one week, and then are cut into sections by microtome.

Observations

Among 975 ovules, only three abnormal embryo sacs, possessing double egg apparatus, were found (one case in variety *Saitama No. 27*, and two cases in *Nōrin No. 42*). The typical structures of normal and abnormal embryo sacs are shown in Figs. 1 and 2, respectively. E_1 and E_2 in Fig. 1 are thought to be egg cell, and no difference was found between egg cell in normal embryo sac and abnormal one. Adjacent to these egg cells, four synergids (S_1, S_2, S_3 and S_4 in Fig. 1) are observed, and two of them (S_1 and S_2), located in micropylar side, have already degenerated at flowering time. It is noticeable that these six cells seem to make up two sets, composed of one egg cell, one normal synergid and one degenerated synergid (E_1-S_3-S_1 and E_2-S_4-S_2), i.e. two sets of egg apparatus exist together in one embryo sac. Polar nuclei in normal embryo sac of wheat, are in close contact with each other, and they never fuse before fertilization. The boundary nuclear membrane between both polar nuclei which locate near the double egg apparatus is, however, not so distinct that two polar nuclei appear to have fused each other to make the so-called central nucleus, but two large nucleoli can be clearly seen separately in it (*P* in Fig. 1). Concerning to the shape and the number of antipodal cells, there is scarcely any difference between two types of the mature embryo sacs. One of such abnormal embryo sacs is shown in Figs. 3 and 4, and another instance is in Fig. 5 in photomicrographs.

---

* Faculty of Agriculture, University of Tokyo, Hongo, Tokyo, Japan.

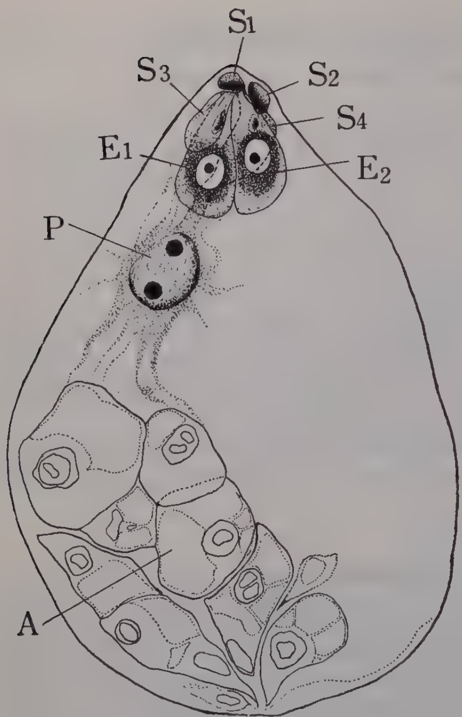


Fig. 1. The embryo sac containing double egg apparatus in *Triticum aestivum* L. (× 180).

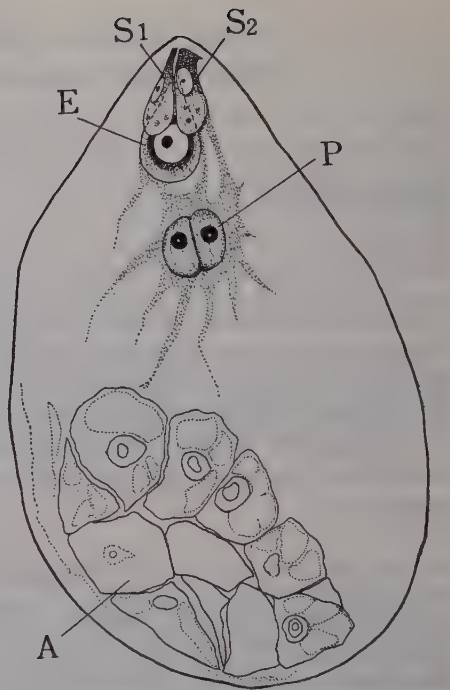


Fig. 2. Normal embryo sac in *T. aestivum* L. (× 180).

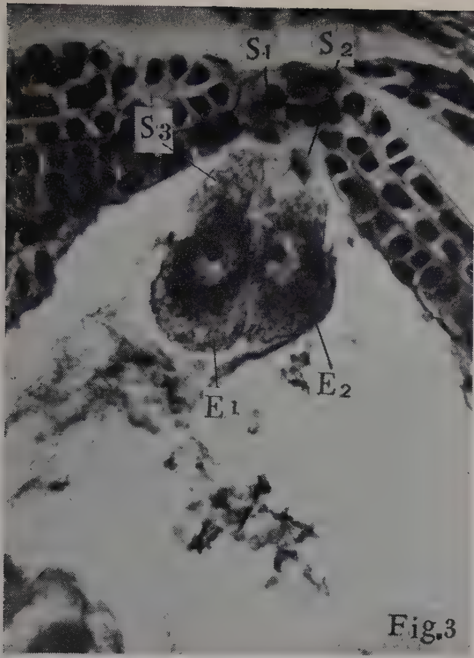
S: synergid, E: egg cell, P: polar nucleus, A: antipodal cell.

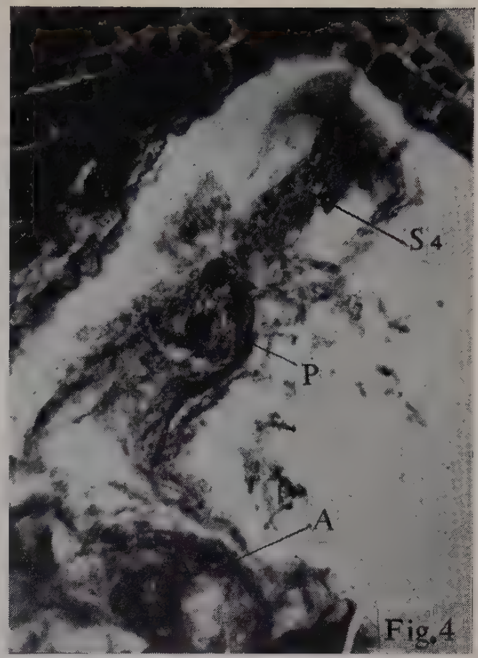
Discussion

Formation of abnormal embryo sac:

It seems necessary to discuss the developmental aspect of embryo sac, to understand the origin of afore-mentioned abnormal embryo sac.

Embryo sac formation of wheat belongs to monosporic type, and according to Percival (1921)[2]), a megaspore gives rise to four nuclei in micropylar end of embryo sac through three times of successive nuclear divisions. Normally, it seems that the rhythm of nuclear division is stopped and the formation of cell membrane occurs around these nuclei, so that one egg cell and two synergids are formed. One nucleus which locates at the most antipodal side of the quartet, is thrown out toward the central portion of embryo sac without the cell membrane formation. It may meet with another nucleus migrated from nuclear quartet in the antipodal side of the embryo sac, and form the polar nuclei. If the rhythm of nuclear division of megagametegenesis is not stopped after its third division due to some reasons, then the fourth division will take place, consequently, resulting in the double states of the egg apparatus, that is: two egg cells and four synergids. The polar nuclei formed after the third nuclear division, can not complete their fourth division, but merely shows weak indication to divide through the disappearance of boundary nuclear membrane. Hence, it is thought that the polar nuclei show the half fuse appearance. The degeneration of two synergids out of four may be due to nutritional or some other causes.





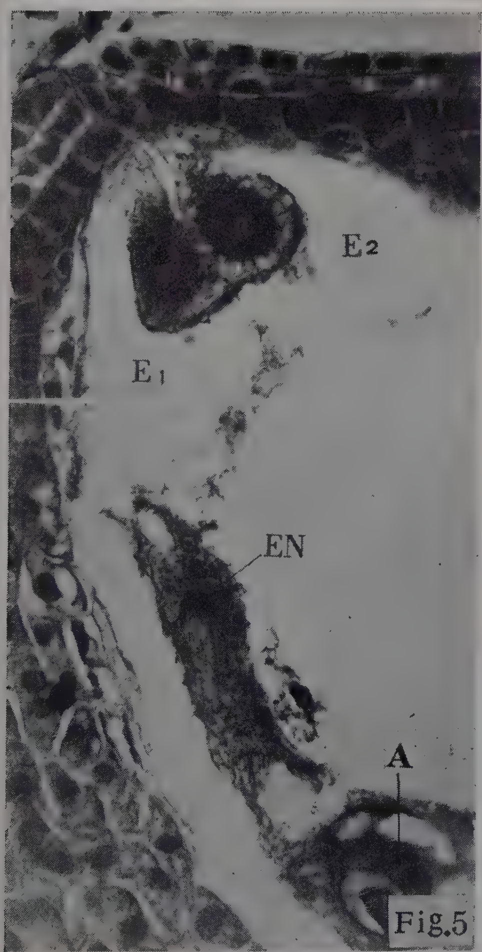


Fig. 3 and 4: Two adjacent sections cut vertically of an embryo sac possessing the double egg apparatus in wheat (Variety *Saitama No. 27.*).

Duplicated egg cells (E_1 and E_2), and three out of four synergids (S_1, S_2 and S_3) are visible in Fig. 3, and another synergid (S_4) and half-fused polar nuclei (*P*) are seen in Fig. 4. The lower parts of both figures are antipodal cells (*A*). (Both × 400).

Fig. 5: Longitudinal section of another embryo sac possessing the double egg apparatus in wheat (Variety *Nōrin No. 42.*)

One of the egg cells (E_1) is already fertilized, but another egg cell (E_2) is as yet unfertilized. Polar nuclei have proceeded already in metaphase of the first nuclei division as the primary endosperm nucleus. (*EN*). One of the degenerating antipodal cells (*A*) is seen in lower part. (× 400).

Watkins (1925)[3]) noticed in hybrid plant between *Triticum turgidum* and *T. vulgare*, though one instance, an embryo sac containing two polar nuclei in contact, three synergids and two egg cells. The present author thinks that Watkins perhaps did not notice another degenerated synergid, however, it cannot be asserted positively, for report lacked in the detailed illustration and discussion of such abnormal embryo sac. In *Crassula shmidtii* and *Umbilicus intermedius*, Mauritzon (1933)[4]) reported that occasionally there is a fourth division in the embryo sac, resulting in the formation of sixteen nuclei, which organize to form four synergids, two egg cells, six antipodal cells and four polar nuclei.

Polyembryony:

In case that a pollen tube enters such abnormal embryo sac, the tip of the pollen tube may penetrate into one of the normal synergids, thus two male nuclei are discharged into embryo sac and are delivered to one of the egg cells and polar nuclei. This inference is drawn from the observations on the fertilization in normal embryo sac.[5]) If an additional pollen tube directly follows the first pollen tube, it can fertilize another egg cell probably by working as agent of a surviving synergid. And that, if these two fertilized egg cells develop completely, the polyembryony or twin embryos with diploid-diploid chromosome numbers will be brought about.

The frequency of the occurrence of embryo sac containing double egg apparatus, is about 1/300 (3 among 978 samples), and there may be little chance of simultaneous arrival of two or more pollen tubes at such egg apparatus, therefore, the fertilization in both egg cells does not always occur.

When only one pollen tube gets into such an embryo sac, there appears usually a normal seed containing one embryo. But if an unfertilized egg cell may also develop parthenogenetically under an impetus from developing fertilized embryo, diploid-haploid twin embryos may be given rise to. In Fig. 5, it is observed that both the egg cell and polar nuclei have finished their fertilization, and the primary endosperm nucleus has already reached the metaphase stage of its first division, whereas another egg cell (E_2) is still lying at unfertilized stage.

On the contrary, another possibility is that two male nuclei penetrate into two egg cells severally without fertilizing to the polar nuclei. This case is also supposed as a source of twin embryos with diploid-diploid chromosome numbers. But if the unfertilized polar nuclei degenerate without development as endosperm, this ovule could not give rise to normal seed grain.

Twin seedlings of wheat has attracted much attention ever since the first report by Namikawa and Kawakami (1934)[6]). In 1954 Nishiyama[7]) gave a thorough review of polyembryony in wheats, including the origins, chromosome numbers and their frequencies of occurrence. According to his review, the frequency of polyembryonic seedling is 0.11 percent, and concerning the chromosome numbers, 2n:2n twins accounts for 84 percent of all twins, and other twins i.e. 2n:n, 2n:3n, 2n:4n etc. and triplets are found quite rarely.

The causes of polyembryony in wheat, like other plants, are interpreted by many workers mainly as follows: the possible and simplest method is "cleavaging" where the proembryo is separated into two or more portions (the so-called identical twins) during its development, hence their chromosomes are 2n:2n (Kappert, 1933[8]), Müntzing, 1937[9])). On the other hand, it is known that the polyembryos are caused through plural origins. For example, Kappert (1933)[8]) explained that the diploid member of 2n:n twins in *Linum ustatissimum* may be derived from fertilized egg cell

and the haploid member from an unfertilized cell of the same embryo sac other than egg cell by haploid parthenogenesis. Kihara (1936)[10]) presented another possibility of 2n:n twins in wheat that diploid embryo may be derived from incidental fertilization of one of the synergids and haploid embryo from unfertilized egg cell. This opinion is based upon the observations made by Watkins (1925)[3]) and Wakakuwa (1934)[11]), who reported the occasional fertilization of synergid by a male nucleus in wheat. Kawakami and Hatamura (1952)[12]) also stated that two synergids of wheat embryo sac are able to fertilize respectively with a male gamete derived from additional pollen tube. The results of present author, however, differ from the aboves, because in spite of about a thousand observation of fertilization examined, not a single case of synergid fertilization by a male nucleus was detected. Kihara[10]) also pointed out a source of 3n:2n polyembryony. He mentioned that one of the endosperm nuclei develops to the triploid embryo additionally to the normal diploid embryo. Besides these, although less frequent, there are two or more embryo sacs in an ovule, and each of which may give rise to embryo (Bacchi 1943[13]) in *Citrus paradisi* Macf. and *C. aurantium* L., and Nielsen 1946[14]) in *Poa pratensis* L. *etc.*). Proliferation of the nucellus or integument cells also may bring about the polyembryony in *Citrus*, *Poa* and other plants.

In addition to these sources, above-mentioned embryo sac containing double egg apparatus, may also be thought as a source of the polyembryony.

Summary

Occurrence of abnormal embryo sac with double egg apparatus, though rarely, was observed in ovules of common wheat, *Triticum aestivum* L. The frequency of its occurrence is about one-three hundredth.

The embryo sac possesses two egg cells and four synergids, and polar nuclei seem to half fuse with each other before fertilization.

Regarding to the process of the embryo sac formation, it is supposed that such double egg apparatus appears through the fourth nuclear division of megaspore in micropylar nuclear group.

This embryo sac with double egg apparatus may be thought to be a new source of polyembryony.

Acknowledgement

The author wishes to express his appreciation to Dr. Y. Togari, Professor of Agronomy, University of Tokyo, for reading the manuscript and for his many valuable comments and criticisms.

References

1) Hoshikawa, K., Agri. and Hort. (Tokyo) **34**: 1143 (1959). 2) Percival, J., Wheat Plant, A Monograph. (1921). 3) Watkins, A. E., Jour. Gen. **15**: 323 (1925). 4) Mauritzon, J., Diss. Lund. 1933. (cited from Maheshwari, P., An Introduction to the Embryology in Angiosperms. 1950). 5) Hoshikawa, K., Proc. Crop Sci. Soc. Japan **28**: 142 (1959). 6) Namikawa, S., and J. Kawakami, Proc. Imp. Acad. **10**: 668 (1934). 7) Nishiyama, I., In Kihara, H.Studies on Wheat Plant. p. 580 (1954). 8) Kappert, H., Biol. Zentralb. **53**: 276 (1933). 9) Müntzing, A., Cytologia, Fujii Jubl. Vol.: 211 (1937). 10) Kihara, H., Agri. and Hort. (Tokyo) **11**: 1425 (1936). 11) Wakakuwa, S., Jap. Jour. Bot. **7**: 151 (1934). 12) Kawakami, J., and M. Hatamura, Jap. Jour. Breed. **1**: 189 (1952). 13) Bacchi, O., Bot. Gaz. **105**: 221 (1943). 14) Nielsen, E. L., Bot. Gaz. **108**: 41 (1946).

摘　　要

普通小麦に見られる二組の卵装置をもつ胚嚢について

星　川　清　親

普通小麦（コムギ）において，一つの胚嚢内に2組の卵装置を有するものが約 1000 箇の胚嚢観察のうち3例見出された．この胚嚢は正常のものに比してやや幅が太く，珠孔側に相同形の2箇の卵細胞が隣接して存在し，その周囲に4箇の助胎細胞（うち最も珠孔側の2箇は小型で退化している）が認められる．また極核は境界の核膜が消失し，なかば融合の様相を呈している．

一般の正常な胚嚢形成に際しては，胚嚢細胞の初回の核分裂で珠孔側に位置した1の核がさらに第2，第3回の核分裂を行なった後，分裂は停止し細胞膜形成が行なわれて1組の卵装置が形成される．これに対し珠孔側でさらに第4回目の分裂が行なわれたために核が倍増されて複卵装置になったものと推察される．また極核においては第4回目の核分裂が弱く不完全で，核膜消失の過程で留まってしまったものと解釈されよう．

従来，双胚種子の起因としていろいろの説明がなされているが，1 つの胚嚢内に2つの卵細胞が存在するという事実から考えて，2 卵細胞がともに発生して双胚を形成することもまた，1 つの双胚の起因として考えられるであろう．因に，小麦においては双胚種子の頻度は約 1/1000 であるが二組の卵装置をもつ胚嚢の頻度は約 1/300 であつた．（東京大学農学部作物学研究室）

Aspergillus niger および *Aspergillus awamori* の醱酵と発芽に関する生理的研究

I. クロカビの浸透価と酸生成との関係

高　見　　亘*

Wataru TAKAMI*: Physiological Studies of *Aspergillus* Connected with Fermentation and Germination. I. Relation between Osmotic Value and Acid Production in *Aspergillus niger* and *Aspergillus awamori*.

1959 年 7 月 10 日受付

クロカビの酸生成の化学的研究については多くの研究があり[1,2]，菌学的研究もなされているが[3]，細胞生理学的研究はほとんどなされていない．筆者は前報[4]でクロカビとパン酵母について，細胞の浸透価が醱酵期間中にいちじるしく変動することを示し，酸生成と密接に関係することを想定したが，pH を変化させた場合以外には十分な検討をしなかった．ここには，クエン酸醱酵において培地の成分を変えた場合，老衰した胞子やナイトロジェン・マスタードによる変異菌を使った場合，シュウ酸，グルコン酸醱酵の場合，Moyer[5]によって発見されたメタノール添加の場合などについて，浸透価と酸生成との関係を報告する．

材料と方法

使用した菌株は当大学でクエン酸醱酵研究用に分離した No. 163 (1956 年 5 月分離) と No. 2078 (1958 年7月分離) とである．クエン酸醱酵の基本培地の組成は次のようである．

しょ糖	140 g
NH_4NO_3	2 g
KH_2PO_4	2 g
$MgSO_4 \cdot 7H_2O$	0.25 g
$MnSO_4 \cdot 4H_2O$	0.02 g
$FeCl_3 \cdot 6H_2O$	0.024 g
麦芽汁	20 cc
蒸溜水	1000 cc

塩酸を以て pH 2.6 に調節．なお，No. 163 で，醱酵中培地をより酸性にする方が好結果がえられる場合には NH_4NO_3 2 g の代りに NH_4Cl 2.7 g を使ったこともある．

シュウ酸醱酵の培地の組成は次のようである．

しょ糖	140 g
$NaNO_3$	4.25 g
KH_2PO_4	3 g
$MgSO_4 \cdot 7H_2O$	0.25 g
$MnSO_4 \cdot 4H_2O$	0.02 g
$ZnSO_4 \cdot 7H_2O$	0.02 g
麦芽汁	20 cc
$FeCl_3 \cdot 6H_2O$	0.024 g
蒸溜水	1000 cc (pH 5.8)

グルコン酸醱酵の培地の組成は次のようである．

ぶどう糖	250 g
$NaNO_3$	30 g
KH_2PO_4	0.3 g
$MgSO_4 \cdot 7H_2O$	0.25 g
蒸溜水	1000 cc

(または $CaCO_3$ 5% 添加)

上記の培養液をクエン酸の深部培養の場合には 500 cc 振盪フラスコに 100 cc ずつ分注し，3白金耳の胞子（または殺菌水 0.5 cc 中に3白金耳の胞子が含まれるように懸濁したもの）を接種，8～10日間 30° で毎分約 110 回転で振盪し，測定のため2日毎に数 cc ずつ取った．（または，振盪フラスコに 80 cc ずつ分注して，振盪機から毎回2個ずつのフラスコを取り除いて測定した）．表面培養の場合には，100 cc フラスコに 30 cc ずつ分注して，1白金耳の胞子を接種，30° で培養し，測定のため毎回2個ずつのフラスコを取り出した．使用菌株

* 早稲田大学生物学教室　Biological Institute, Waseda University, Tokyo, Japan.

では，ペンタブロムアセトン法によってクエン酸の定量をしてみると全酸の 98% 程度がクエン酸であることが知られたので，Doelger & Prescott[6]) にしたがって2日目ごとに 2cc の培養液をとり，フェノールフタレインを指示薬として0.1N NaOH によって中和し，これを 10cc に換算した滴定酸度を求めた．シュウ酸はバウ氏液によって定性的に調べ，グルコン酸は培養液 5cc をとり，$CaCO_3$ を添加しない場合には消石灰で培養液を中和し，それを50%アルコールで沈でんさせグルコン酸カルシウムとし，105°，4時間乾燥後秤量した．

菌体の浸透価は菌糸をしょ糖液に浸すとすぐに原形質分離をすることがわかったので，菌糸をスライドガラスにとり，しょ糖液を注いで ×1200 程度で検鏡して求めた．ナイトロジェン・マスタード処理は有馬，阿部[7]) のと同じである．

実験結果

1. 基本培地におけるクエン酸醱酵： 上記の培地を 500cc 振盪フラスコ3個に 100cc ずつ分注し，前培養の接種後5日目の No. 2078 の胞子を 0.5cc 中に3白金耳の胞子が含まれるように殺菌水に懸濁し，その 0.5cc ずつを接種，2日毎に数 cc ずつをとって滴定酸度と浸透価を測った結果は表1のようであり，基本培地ではフラスコによる滴定酸度のフレは5以下としてよく，浸透価にフレはないと考えられる．1フラスコについて3白金耳ずつの胞子を接種すると滴定酸度のフレはも少し大きくなるが多くとも 10 以下であるとしてよく，この表の結果から浸透価が 1.2 モル程度になるとクエン酸の生成が盛んになることがわかる．

Table 1. Titratable acidity and osmotic value in Strain 2078 in control medium.

Expt. \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of broth) 2	4	6	8	10
No. 1	0.5	11.0	37.3	52.8	68.8
2	0.5	10.5	38.3	53.0	69.8
3	0.5	11.5	38.7	56.3	75.4

Expt. \ Days	Osmotic value (M) 2	4	6	8	10
No. 1–3	1.6	1.4	1.2	1.0	1.0

2. クエン酸醱酵における窒素濃度： NH_4NO_3 の濃度だけを 1l につき 1, 2, 3g と変えた3種の培地を 500cc 振盪フラスコ各2本に 100cc ずつ分注し，No. 2078 の5日目の胞子の懸濁液を上記のように接種，2日毎に数 cc ずつとして滴定酸度と浸透価を測った結果は表2のようである．また，NH_4Cl の濃度だけを 1l につき 1, 3, 5g と変えた3種の培地に，No. 163 の約1週間後の胞子を接種した場合の結果は表3のようである．どちらの場合でも窒素の濃度が小さい場合にはクエン酸の最終生成量は少なく，No. 2078 では 2〜4 日のクエン酸生成量は control より多いが，後には少なくなり，浸透価は control に比べてさがらないで 1.3 に止まったが，より古く分離された No. 163 では窒素の濃度が小さい場合には control に比べて浸透

Table 2. Effect of concentration of NH_4NO_3 on titratable acidity and osmotic value in Strain 2078. The spores were inoculated in the media containing NH_4NO_3 of three different concentrations after 5 days' preculture.

Conc. of NH_4NO_3 \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of broth) 2	4	6	8	10
1 g/l	3.3	16.3	35.4	41.0	49.1
2 g/l	0.5	11.0	38.1	54.0	71.3
3 g/l	2.6	13.1	22.9	44.6	70.9

Conc. of NH_4NO_3 \ Days	Osmotic value (M) 2	4	6	8	10
1 g/l	1.5	1.4	1.3	1.3	1.3
2 g/l	1.6	1.4	1.2	1.0	1.0
3 g/l	1.6	1.4	1.4	1.3	1.0

Table 3. Effect of concentration of NH_4Cl on titratable acidity and osmotic value in Strain 163. The spores were inoculated in the media after 7 days' preculture.

Donc. of NH_4Cl \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of broth) 2	4	6	8	10
1 g/l	0.8	3.6	6.1	10.5	10.5
3 g/l	5.3	8.7	29.8	50.8	62.5
5 g/l	5.4	10.5	28.4	43.6	54.1

Conc. of NH_4Cl \ Days	Osmotic value (M) 2	4	6	8	10
1 g/l	1.5	1.0	0.9	0.8	0.8
3 g/l	1.6	1.2	1.2	1.2	1.2
5 g/l	1.7	1.3	1.2	1.2	1.2

価の下降が著しく，クエン酸生成量は甚だ少なかった．なお，培地を各フラスコに 80 cc ずつ分注して，No. 2078 の 10 日後の胞子を接種し，2日毎に各種につき2個ずつのフラスコを振盪機から取去り，それについて調べた結果は表4のようで，1 g/l の方がクエン酸生成が多く，4 g/l では著しい害作用がみられ，浸透価も 1.4 モルよりさがらなかった．

Table 4. Effect of concentration of NH_4NO_3 on titratable acidity and osmotic value in Strain 2078. The spores were inoculated in the media after 10 days' preculture. In this case measurements were made per 2 flasks every 2 days.

Conc. of NH_4NO_3 \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of borth) 2	4	6	8
1 g/l	0.4	21.0	46.2	59.4
2 g/l	1.6	12.6	35.7	55.2
4 g/l	3.2	8.4	12.1	15.5

Conc. of NH_4NO_3 \ Days	Osmotic value (M) 2	4	6	8
1 g/l	1.4	1.2	1.0	1.0
2 g/l	1.6	1.4	1.2	1.0
4 g/l	1.7	1.4	1.4	1.4

Table 5. The results of titratable acidity and osmotic value in Strain 2078 when the concentration of NH_4NO_3 in the medium was changed on the 3rd day. (Initial concentration of No. 1), 2), 3) is 1.0, 1.3, 1.6% respectively and is increased to 2% on the 3rd day.)

Experiments \ Days	Titratable acidity 3	8	Osmotic value 3	8
Control (2%)	8.0	26.0	1.5	1.3
No. 1	9.2	38.3	1.3	1.1
No. 2	10.0	38.0	1.3	1.1
No. 3	9.0	27.0	1.4	1.3

次に NH_4NO_3 だけを 1.0, 1.3, 1.6%と変えた3種の培地各 40cc に接種し，3日間培養した後に NH_4NO_3 の 40 cc を加えてどの濃度も2%になるように加減すると表5に示すように，control よりかによい酸生成がえられ，浸透価もそれに応じて変はるったことが認められた．

3. クエン酸酵における金属イオン濃度: KH_2PO_4 の濃度だけを 1 l につき 1, 2, 3 g と変えた3種の培地に，上記のように No. 2078 の5日目の胞子を殺菌水に懸濁して接種，2日毎に数 cc ずつとって調べた結果が表6で，3 g/l の場合に害作用がわずかに見られる．表7は No. 163 の約2週間後の胞子を KH_2PO_4 の濃度だけを 1 l につき 1, 2, 4 g と変えた培地に接種した場合の結果で，control のクエン酸生成も少なかったが，Kの欠乏も過剰も悪影響が大きく，K欠乏の場合には誘導期と成長期にも浸透価が小さく，正常でないことを示している．また，この No. 163 のK過剰の場合に限ってシュウ酸が併生した．

Table 6. Effect of concentration of KH_2PO_4 on titratable acidity and osmotic value in Strain 2078. The spores were inoculated in the media after 5 days' preculture.

Conc. of KH_2PO_4 \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of broth) 2	4	6	8	10
1 g/l	0.6	7.9	39.9	55.7	76.1
2 g/l	0.5	11.0	38.1	54.0	71.3
3 g/l	2.6	8.4	24.2	48.1	67.5

Conc. of KH_2PO_4 \ Days	Osmotic value (M) 2	4	6	8	10
1 g/l	1.5	1.2	1.0	1.0	1.0
2 g/l	1.6	1.4	1.2	1.0	1.0
3 g/l	1.5	1.4	1.4	1.2	1.1

Table 7. Effect of concentration of KH_2PO_4 on titratable acidity and osmotic value in Strain 163. The spores were inoculated in the media after about 2 weeks' preculture.

Conc. of KH_2PO_4 \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of broth) 2	4	6	8	10
1 g/l	2.5	8.9	14.6	19.4	21.0
2 g/l	3.7	14.0	19.7	23.7	33.1
4 g/l	4.6	8.8	11.7	14.5	18.9

Conc. of KH_2PO_4 \ Days	Osmotic value (M) 2	4	6	8	10
1 g/l	1.0	1.1	1.1	1.1	1.1
2 g/l	1.6	1.4	1.2	1.2	1.2
4 g/l	1.6	1.3	1.3	1.3	1.3

次に，$MgSO_4$ 濃度だけを 1 l につき 0.125, 0.25, 0.5 g と変えた3種の培地に No. 2078 の4日目の胞子を殺菌水に懸濁して接種，2日毎に数cc ずつとって調べた結果が表8で，胞子の年令がちが

Table 8. Effect of concentration of $MgSO_4$ on titratable acidity and osmotic value in Strain 2078. The spores were inoculated in the media after 4 days' preculture.

Conc. of $MgSO_4$ \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of broth) 2	4	6	8	10
0.125 g/l (a)	6.4	12.3	28.9	69.6	76.5
0.125 g/l (b)	2.1	11.8	27.2	61.5	68.5
0.250 g/l	0.5	8.6	19.8	50.2	57.2
0.500 g/l (a)	0.0	9.1	25.5	67.6	82.4
0.500 g/l (b)	0.0	5.9	14.7	43.7	60.5

Conc. of $MgSO_4$ \ Days	Osmotic value (M) 2	4	6	8	10
0.125 g/l (a)	1.0	0.9	0.8	0.8	0.8
0.125 g/l (b)	0.9	0.9	0.8	0.8	0.8
0.250 g/l	1.5	1.3	1.2	1.2	1.2
0.500 g/l (a)	1.5	1.4	1.1	0.9	0.9
0.500 g/l (b)	1.5	1.4	1.3	1.3	1.3

うためか control のクエン酸生成量は表1のものより少なかったが，Mg の濃度が大きい場合に浸透価と滴定酸度のフレがみられた．表9は No. 163 の約2週間後の胞子についての同様な実験の結果である．この場合には Mg の濃度が小さいときには菌糸ができにくく，その幅は control の8割程度で，内容物も少なかった．

Table 9. Effect of concentration of $MgSO_4$ on titratable acidity and osmotic value in Strain 163. The spores were inoculated in the same media as in Table 8 after about 2 weeks' preculture.

Conc. of $MgSO_4$ \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of broth) 2	4	6	8	10
0.125 g/l	4.3	13.8	16.7	22.1	22.1
0.250 g/l	4.0	15.8	24.6	27.5	30.2
0.500 g/l	4.3	14.3	25.0	28.0	32.9

Conc. of $MgSO_4$ \ Days	Osmotic value (M) 2	4	6	8	10
0.125 g/l	1.6	1.4	1.2	1.2	1.2
0.250 g/l	1.6	1.4	1.2	1.2	1.2
0.500 g/l	1.5	1.3	1.2	1.2	1.2

表 10 は $FeCl_3$ の濃度だけを 1 l につき 0.012, 0.024, 0.048 g と変えた3種の培地に No. 2078 の4日目の胞子を殺菌水に懸濁して接種，2日毎に数 cc ずつとって調べた結果で，Mg の場合のように Fe の濃度の変化によるフレがあり，濃度が control の2倍になると阻害と促進の両方がみられたが，No. 163 では Fe の濃度が大きいと害作用が著しかった．

Table 10. Effect of concentration of $FeCl_3$ on titratable acidity and osmotic value in Strain 2078. The spores were inoculated in the media after 4 days' preculture.

Conc. of $FeCl_3$ \ Days	Titratable acidity (0.1 N–NaOH cc/10 cc of broth) 2	4	6	8.5	10
0.012 g/l (a)	0.0	7.0	14.4	46.5	51.9
0.012 g/l (b)	1.8	6.6	23.8	61.0	72.5
0.024 g/l	0.5	8.6	19.8	50.2	57.2
0.048 g/l (a)	2.1	12.8	18.8	52.4	65.3
0.048 g/l (b)	0.0	5.1	16.6	40.7	46.9

Conc. of $FeCl_3$ \ Days	Osmotic value (M) 2	4	6	8.5	10
0.012 g/l (a)	1.5	1.3	1.3	1.3	1.3
0.012 g/l (b)	1.5	1.3	1.1	1.0	1.0
0.024 g/l	1.5	1.3	1.2	1.2	1.2
0.048 g/l (a)	1.5	1.4	1.3	1.1	1.0
0.048 g/l (b)	1.5	1.4	1.2	1.4	1.4

4. クエン酸醱酵におけるしょ糖濃度： しょ糖の濃度だけを 8，14，20% と変えた培地に No. 2078 の約1週間後の胞子と No. 154 の前培養しない保存胞子を接種，2日毎に各種につき2個ずつのフラスコを振盪機から取り去り，それについて調べた結果が表 11 で，表 12 は No. 163 の約2週間後の

Table 11. Effect of concentration of sucrose on titratable acidity and osmotic value in Strain 2078 and Strain 154. Two flasks were removed for observation every two days.

Conc. of sucrose \ Days	Titratable acidity (0.1 N-NaOH cc/10 cc of broth) 2	4	6	8
8%*	6.6	13.5	33.6	47.3
14%*	1.6	12.6	35.7	55.2
20%*	6.5	7.3	35.2	35.4
14%**	0.0	2.6	8.7	10.5

Conc. of sucrose \ Days	Osmotic value (M) 2	4	6	8
8%*	1.6	1.4	1.2	1.0
14%*	1.6	1.4	1.2	1.0
20%*	1.6	1.4	1.2	1.0
14%**	0.8	1.0	1.1	0.8

* Strain 2078, the spores were inoculated in the media after 1 week's preculture.
** Strain 154, not precultured.

Table 12. Effect of concentration of sucrose on titratable acidity and osmotic value in Strain 163. The spores were inoculated in the media after about 2 week's preculture.

Conc. of sucrose \ Days	Titratable acidity (0.1 N-NaOH cc/10 cc of broth) 2	4	6	8
2%	3.2	10.5	11.3	17.3
6%	4.3	15.2	19.0	25.7
10%	5.3	12.8	23.7	29.6
14%	5.6	16.8	35.1	38.9

Conc. of sucrose \ Days	Osmotic value (M) 2	4	6	8
2%	1.4	1.0	1.0	0.9
6%	1.4	1.0	1.0	0.9
10%	1.5	1.2	1.1	1.1
14%	1.6	1.3	1.2	1.1

胞子について，2日毎に数 cc ずつとって調べた結果である．しょ糖濃度のちがいによってクエン酸の生成量がちがうにもかかわらず浸透価のちがいは前の諸項の場合程著しくないようで，前培養しない古い胞子では，醱酵過程の誘導期，生長期のような初期にも浸透価は低く，クエン酸の生成量も甚だ少なかった．

5. ナイトロジェン・マスタード処理の影響： 菌株 154 S 63 についてナイトロジェン・マスタード

Table 13. Effect of nitrogen mustard treatment on titrattable acidity and osmotic value in Strain 154 S 63.

Mutants \ Days	Titratable Acidity (0.1 N-NaOH cc/10 cc of broth) 1	7
Parent strain	0.6	18.3
Cinnamon type[1]	0.2	25.9
Brown tyye[2]	0.2	17.3
Sterile type[3]	0.3	6.4
〃 〃	0.2	3.2

Mutants \ Days	Osmotic value (M) 1	10
Parent strain	1.5	1.0
Cinnamon type[1]	1.2	1.0
Brown type[2]	0.8	1.0
Sterile type[3]	*	*
〃 〃	*	*

* already plasmolysed

1 Cinnamon colored conidia, 2 Brown colored conidia, 3 Sporulation was repressed remarkably or completely.

処理による変異がえられたが[8]，そのうちの数種について滴定酸度と浸透価を調べた結果が表 13 で，変異菌では最初の浸透価も低いようで，なかには発芽の最初から原形質分離しているものもあった．なお，この実験では親株より著しく多くクエン酸を生成するものはえられなかった．

Table 14. Effect of MeOH on titratable acidity and osmotic value in Strain 2078 surface-cultured.

Conc. of MeOH \ Days	Titratable acidity (0.1 N-NaOH cc/10 cc of broth) 2	4	6	8
0%	2.6	8.4	14.8	26.3
1%	0.5	10.5	44.6	87.2
3%	0.0	9.9	27.8	84.5

Conc. of MeOH \ Days	Osmotic value (M) 2	4	6	8
0%	1.6	1.5	1.4	1.4
1%	1.6	1.5	1.3	1.2
3%	*	1.7	1.4	1.2

* not germinated

6. クエン酸醱酵におけるメタノール等添加の影響： No. 2078 の4日目の胞子の場合にはメタノールを1%添加しても害作用がみられたが胞子の年令が2週間後のものでは，表面培養したところ表 14 のような促進作用がみられ，クエン酸の生成量が多いにもかかわらず最終浸透価は表 1，2 などに比べて大きい．なお，この場合に，メタノールによって発芽と初期の生長が阻害されるので菌糸の形は花粉の発芽が不良の場合[9]のようになる（図1）．表 15 は，No. 163 を表面培養法で，培地にメタノールのほかに，寒天，バレイショ，でんぷんをそれぞれ 0.5%加えて培養した結果で，この場合にもメタノール添加の最終浸透価は control よりも大きく，メタノールだけ添加の場合にはややもすると菌糸が沈みがちであるが，さらに粘性物質を加えるとそれが防げて酸生成は著しく増加し，最終浸透価も

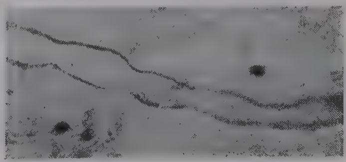


Fig. 1. The irregular form of the hypha of *Aspergillus niger* in methanol containing medium. ×1000.

Table 15. Effect of agar or strach plus MeOH on titratable acidity and osmotic value in Strain 163 surface-cultured. Two flasks were removed for observation every two days.

Addition \ Days	Titratable acidity (0.1 N-NaOH cc/10 cc of broth) 2	4	6	8	10
Blank	5.0	13.0	23.7	28.2	35.2
MeOH 3%	2.7	27.5	31.5	45.5	62.5
Agar 0.5%	6.2	17.2	25.2	23.3	24.1
Starch 0.5%	9.8	7.6	9.6	8.2	7.5
Agar & MeOH	5.5	27.9	66.8	86.4	89.8
Starch & MeOH	9.9	23.8	65.3	79.9	83.2

Addition \ Days	Osmotic value (M) 2	4	6	8	10
Blank	1.6	1.3	1.0	0.9	0.8
MeOH 3%	1.7	1.6	1.3	1.0	1.0
Agar 0.5%	1.6	1.3	1.0	0.9	0.9
Starch 0.5%	1.6	1.3	0.9	0.8	0.8
Agar & MeOH	1.8	1.6	1.4	1.3	1.1
Starch & MeOH	1.8	1.6	1.4	1.3	1.1

いくらか大きかった.

7. シュウ酸とグルコン酸醱酵: No. 163 を表面培養法によってシュウ酸醱酵させた場合には2日後にすでに菌蓋が厚く，浸透価もすぐさがってシュウ酸が多量に発生し，表 16 に示すようになった．また，No. 2078 を振盪培養法によってグルコン酸醱酵をさせた結果は表 17 に示すようで，6日ごろから酸生成が高くなり，浸透価はさがった.

Table 16. Osmotic value and weight of mycelium in oxalic fermentation in Strain 163.

Days	1	3	5	7	9	12
Osmotic value (M)	1.6	0.9	0.9	0.9	0.9	0.8
Wt. of mycelium (g)	—	0.622	0.549	0.747	0.590	0.740

Table 17. Gluconic fermentation and osmotic value in Strain 2078.

Days	2	4	6	8
Gluconic acid (g)	0	0.11	0.13	0.27
Osmotic value (M)	1.6	1.5	1.4	1.1

考察および結論

上の実験結果によって，前報[4]で述べたように，酸生成が多い場合には，発芽および生長の初期には養分が吸収されて，浸透価は大きく，1.6～1.7 モル程度になり，このときの浸透価が小さすぎるものは酸の総生成量も少ない．前培養しない保存胞子やナイトロジェン・マスタード処理をしたものはこの初期の浸透価は甚だ小さく，培地の N や K または Mg の濃度が小さすぎる場合にも初期の浸透価は小となり，2日目のが 1.4 モル以下の場合には一般にその後の酸生成量は少ない．また，クエン酸醱酵の目的は代謝中間物であるから過剰の栄養源はかえって酸生成を不良にするが，著しい阻害を与えるような限界濃度は菌株によってちがうようで，菌株自身の特殊性と分離してからの期日の長短にも関係しよう．

次に，4日目あたりに浸透価が 1.2～1.3 モル程度にさがってくると酵素が合成されるか，または少なくとも活性化され，酸生成が盛んになり醱酵期[10]が始まる．一般的に培地の成分の濃度が小さい場合には醱酵期が早く始まるが，浸透価がどんどんさがり酸の生成も極めて少ないようなものでは，生育相[11]の成長期，定常期も短いことになる．ところが6日後になっても浸透価があまりさがらずに 1.3～1.4 モルに止まるようなものでは定常期は長いが，酸生成は不良で，その浸透価もさがらないようである．このように，1.2 から 1.0 モル前後の間が酸生成が盛んな細胞液の濃度で，この時期[10]が醱酵期といわれている状態である．この実験ではクエン酸醱酵に重点をおいたが，その他の酸生成の場合にも表 16～17 が示すように浸透価はクエン酸生成の場合と同様な変化をするものと考えられる．Mg, Fe の濃度の変化による浸透価や酸生成に対する影響は N, K のそれに比べて確定的ではないようで，しょ糖の濃度の変化が N, K のそれにくらべて浸透価にはそれ程著しい影響を与えないのは透過性のちがいによるものであろう．前報にも示したように糖の消費曲線は5日目頃急増し，したがって最初は 14 %のような多量の炭素源は不要である．このような考察の応用として，上にも一つの実験を述べたが，培養の途中でしょ糖や窒素などの濃度を変える装置の考察は実用上有益なものであろう．

最後に，培地にメタノールを 1～3% 添加すると最初発育が阻害されるが，後にはしだいに control に追いつくような現象が観察され，浸透価や菌糸の

外形にも阻害作用，すなわち生育相のズレがみられ，浸透価は control のものに比べて大きく，成長期が始まるにつれて急にさがり，そのとき酸生成も大いに促進される．表面培養法においては，メタノールのほかに寒天，でんぷんのような粘性物質を培地に添加すると，菌蓋の沈降が防がれ，メタノールの効果はさらに増大することがわかる．

文　献

1) Prescott S.C., and Dun, C.G., Industrial Microbiology (1949). 2) Foster J.W., Chemical Activities of Fungi (1949). 3) Ozaki A., J. Agr. Chem. Soc. Japan **29**: 611 (1955). 4) Takami W., Bot. Mag. Tokyo **70**: 140 (1957). 5) Moyer A.J., Appl. Microbiol. **1**: 1 (1953), U.S.P. 2,674,561 (1954). 6) Doelger W.P. and Prescott, S.C., Ind. Eng. Chem. **26**: 1142 (1934). 7) Arima K. and Abe, S., J. Antibiotics **4**: 342 (1951). 8) Kawate S., Techinology Reports of Kansai Univ. **1**: 45 (1959). 9) Takami W., Bot. Mag. Tokyo **69**: 128 (1956). 10) Shu P., and Johnson, M.T., Ind. Eng. Chem. **40**: 1202 (1948). 11) Monod J., Ann. Rev. Microbiol. **3**: 371 (1949).

Summary

Surveying the relation between the acid (citric, gluconic and oxalic acid) production and the osmotic pressure of the mycelium in many cases, the following results were obtained.

1. In good acid production, the initial osmotic pressure is as high as 1.6-1.7 M.

2. The initial osmotic pressure of the old strain, not pre-cultured and of the nitrogen-mustard treated strain is low.

3. When the osmotic value decreases to about 1.3-1.2 mol, the acid production is accelerated and then increases gradually during the period when the osmotic value is 1.0-0.9 M.

4. Optimum concentration of sucrose in basal medium is 10-14%. At the growth stage 10% of sucrose is preferable, while at the stationary stage, 14%.

5. Effects of deficiency and excess of N and K are remarkable, as compared with those of Mg and Fe. It may be said that as in the case of sucrose, a lower concentration of N is preferable at the grwoth stage and a higher concentration of it at the stationary stage.

6. If 1-3% methanol is added in basal medium, the irregular forms of hyphae are observed at the growth stage and in many cases more citric acid is produced. In such cases, citric acid production is more promoted than at the ordinary osmotic value. If we add agar and starch moreover we have much more citric acid production.

7. As an application of this observation, we can have more citric acid production, when the initial concentration of NH_4NO_3 in basal medium is 1% and increases to 2% on the 3rd day.

サンショウモの日長反応に関する研究
(IV) 日長効果におよぼす酵素阻害剤の影響について

柴　田　　治*

Osamu SHIBATA*: Studies on Photoperiodic Responses of *Salvinia natans*. (IV) The Effects of Certain Enzyme Inhibitors.

1959 年 1 月 22 日受付

花芽形成の際の物質代謝の変化に関してはKraus and Kraybill[1]による C/N 率の提唱以来多くの報告がある．しかし酵素阻害剤を用いた解析的実験は非常に少なく，わずかに Melchers ら[2]と中山[3,4]による報告がみられるにすぎない．

短日植物のアサガオで酵素阻害剤を用いて暗期反応を解析した中山の報告[3]によれば，重金属酵素は暗期反応と密接な関係があり，解糖系は花芽形成に関与しているが，クエン酸回路はこれに関係していないという．

筆者は短日植物のサンショウモを用いて，日長効果におよぼす酵素阻害剤の影響を調べたのでその結果をここに報告する．

材料および方法

実験材料および実験方法はすべて前報[5]と同じである．短日処理は明期 8 時間，暗期 16 時間として 5 回くり返し，この間明期のみ，暗期のみ，または明暗両期を通じて阻害剤を与えた．阻害剤を与える場合は該当期間だけ所定濃度の阻害剤を含む培養液（含毒培養液）に植物を移し，これ以外の時には阻害剤を含まない培養液（無毒培養液）で培養した．

阻害剤としては KCN, NaN_3, CO, KH_2AsO_4, NaF, マロン酸を用いた．明期あるいは暗期にのみ阻害剤を与える場合は，含毒培養液から無毒培養液に植物を移す際の水洗には十分注意した．すなわち，明期にのみ阻害剤を与える場合は，8 時間明期の最後の 20 分間を流水中におき，阻害剤をできるだけ洗い流し，その後無毒培養液に移して暗処理を行なった．したがって明期は 8 時間であっても含毒培養液中に植物がおかれた時間は 7 時間 40 分である．時期にのみ阻害剤を与える場合は 16 時間の暗期後直ちに流水中に移し，明所で 20 分間水洗した後無毒培養液に移した．

短日処理効果の比較は前報[5]と同様に胞子果数に関して求めた阻害度と着果個体率で行ない，さらに胞子果の成形状態も記録した．いずれの場合も対照とした植物は全く阻害剤を与えなかった区のものである．

結　　果

KCN——10^{-4} M の KCN を明期または暗期に与えると，いずれの場合も胞子果形成反応は抑制され，殊に明期に与えた場合顕著な抑制がみられた．すなわち阻害度は明期に KCN を与えた場合 78.8 %，暗期に与えた場合 32.7% であつた．

明期反応阻害の原因としては，光合成の阻害にもどずく炭水化物その他の代謝基質の欠亡と，それ以外の代謝系の阻害が考えられる．そこでつぎの実験では，炭水化物の欠亡を補なうために明期中 10^{-4} M の KCN と同時に 0.5%のブドウ糖を与えて同様の実験を行なった．その結果胞子果形成の阻害度は −32.7%となり，胞子果数は対照区のものよりかなり増加し，その発育もより良好であった．この場合 KCN を与えず，0.5% にブドウ糖のみを与えた植物では対照区のものよりわずかに胞子果の発育が劣っていた．

暗期中 KCN を与えた場合の KCN 濃度と胞子果数の関係を第 1 図に示した．ただし胞子果に関したこれらの数値には成形の不完全な胞子果原基数も算入されている．

* 信州大学文理学部生物学教室　Department of Biology, Faculty of Liberal Arts and Science, Shinshu University, Matsumoto, Japan.

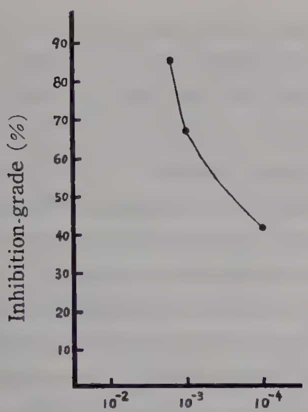


Fig. 1. A relationship between the concentration of KCN added in the dark period and the inhibition-grade.

図示はできなかったが，濃度の変化につれて原基から完全果まで胞子果の発達の程度はかなり異なっていた．濃度は低い程胞子果の発達が良好となり，特に 10^{-5} M と 10^{-6} M の間で著しい違いがみられた．すなわち 10^{-5} M では形成された胞子果の約半数が原基のままであったのに反し，10^{-6} M ではほとんどすべてが完全に成形していた．10^{-6} M 以下の濃度ではすべて完全な胞子果をつけていたが，対照区のものにくらべるとその発育は多少劣っていた．

着果節位による胞子果の成形の程度の違いはそれ程著しくはなかったが，先端部のもの，すなわち遅く形成されたものの方が多少良好であつた．着果個体率はすべての場合に 100%であった．

阻害剤を与えた植物の生長はその処理直後には対照区のものよりわずかに劣っていたが，その後はほとんど差がみられなくなつた．

NaN_3——処理方法は KCN の場合と同様である．結果も KCN の場合と同じ傾向を示したが，生存許容濃度および同程度の阻害を生ずるに必要な濃度は KCN より低かった．たとえば，5×10^{-5} M の NaN_3 を暗期中に与えると着果個体率は 40% 阻害度は 80.8% となり，10^{-5} M の阻害度は明期に与えた場合 55.8% 暗期に与えた場合に 14.4% を示した．10^{-5} M では着果個体率はいずれも 100% となった．

明期に 10^{-5} M の NaN_3 を与えると上述のように胞子果形成はかなり阻害されるが，これと同時に 0.5% のブドウ糖を与えると阻害度は −33.7% となり，KCN の場合と同様に胞子果数は対照区のものより多くなった．

暗期に与えた NaN_3 の濃度と胞子果形成阻害度の関係も KCN の場合と同じ傾向を示した．

CO——この実験では暗期にのみ CO を与えてその影響を調べた．用いた CO の濃度は 90%で残りの 10% は空気である．この濃度はサンショウモの生存許容限界として予備実験できめたもので，長期間これに曝した場合には多少黄化した．しかし以後の生育には全く障害とならず，葉の色も処理後 1〜2 日で正常の緑色に戻った．

暗期中 CO を与えると阻害度は −74%となり，対照区の植物より著しく胞子果数を増した．処理回数の少ない時の方が胞子果数増加の比率は大きくなった．胞子果の発育も対照区のものよりかなり良好で，栄養体の生長もよく，葉は対照区のものに比してずっと大形になった．

KH_2AsO_4——10^{-5} M の KH_2AsO_4 を明暗両期を通じて与えると第 1 表に示すように阻害度は 57.7 %となるが，KH_2AsO_4 と同時に 10^{-4} M の KH_2PO_4 を与えると阻害度は −10.6% となり As 塩と P 塩の拮抗作用がみられた．

Table 1. Effect of arsenate as antagonizing with that of phosphate on the photoperiodic response of *Salvinia natans*.

Applied solutions		Results	
Kinds	Supplied components*	Inhibition-grade (%)	Induced plants (%)
Solution A	10^{-5} M KH_2AsO_4	57.7	85.7
Solution B	10^{-5} M KH_2AsO_4 10^{-4} M KH_2PO_4	−10.6	100
Control	no components	0	100

* Chemical components were dissolved into the culture solution to give a provided concentration.

KH_2AsO_4 処理をした植物で特にめだつことは，胞子果の一部が欠損した形態的に異常なものがかなりの数観察されたことである．As 塩のみを与えた場合には胞子果の発達はきわめて悪く，約半数はやや大形の原基のままであるか，胞子果として分化したものでも形態的には異常であった．これに反し，P 塩が共存した場合には胞子果の形成，発育いずれ

も対照区のものよりわずかながら優れていた．

種々の濃度の KH_2AsO_4 を明暗両期を通じて与えた場合 10^{-3} M では栄養体の発育と胞子果の形成は完全に抑制された．10^{-4} M では胞子果阻害度は 10^{-5} M の場合と大差なかったが，つくられた胞子果の大半は原基のままか発育が不完全であり，栄養体の生長も未だ完全とはいえなかった．しかし 10^{-7} M では阻害度はほぼ 0％となり正常形の胞子果がつくられた．

NaF——NaF も明暗両期を通じて与えた．結果は第2図に示した．着果個体率は NaF 濃度 2×10^{-3} M で 66.7％ となったが，それ以下の濃度では 100％であった．

胞子果の発育は 2×10^{3} M の NaF を与えた場合，非常に悪く，ほとんどが原基のままであった．10^{-3} M ではやや発育良好となったが正常な発育はみられず，10^{-4} M では正常に近い発育を示した．10^{-3}, 10^{-4} M では着果節位が先端部に近い程すなわちおそく分化したもの程完全に成形されていた．

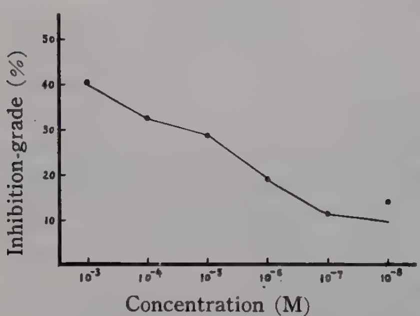


Fig. 2. A relationship between the concentration of NaF added in the inductive period and the inhibition-grade.

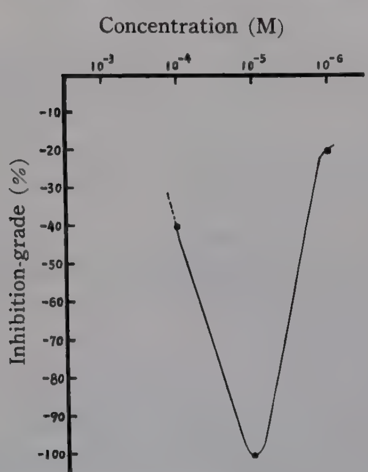


Fig. 3. A relationship between the concentration of malonic acid added in the inductive period and the inhibition-grade. (The plants were perished in 10^{-2} and 10^{-3} M.)

マロン酸——マロン酸も明暗両期を通じて植物に与えた．10^{-2}, 10^{-3} M では植物は褐変して枯れてしまったが，これ以下の濃度ではそのようなことはなかった．これによって試みた濃度範囲（10^{-4}～10^{-6} M）ではすべて胞子果数は対照区のものより増加し，特に 10^{-5} M の場合にその増加は著しく，対照区のものの2倍にも達した（第3図）．

植物の生長はいずれも良好であり 10^{-4}, 10^{-5} M では対照区よりも良いように思われた．しかし胞子果の発育は少しく劣っていた．

考　　察

炭水化物代謝におけるクエン酸回路の関与はすでに多くの動植物で認められているから，以下この一般的な見解にしたがって，サンショウモの炭水化物代謝にもクエン酸回路が関与しているものとして考察を進める．

一般に酵素阻害剤を用いて酵素能や代謝系を調べる場合，阻害剤を除いて酵素能を再活性化するには無細胞の酵素液を用いてもかなり長時間の透析を必要とする．まして多細胞の生体を用いた場合は，阻害剤を除去するのに非常に長時間を要するものと考えられる．光週性の実験に際して明期または暗期に阻害剤を含む液中に葉を浸し，次の暗期または明期のはじめにその葉を入念に水洗しても，わずかな時間で葉中の阻害剤が完全に除去されるとは考え難い．ことに陸生植物ではその後葉は空気中におかれるため，残余の阻害剤の排出は全く不可能であろう．したがって葉を水洗したとしても直ちに酵素阻害作用がなくなるとは考えられない．ここで用いた水生のサンショウモでもこの点についてはかなりの考慮を要する．ただ CO だけは処理後の除去が比較的容易であって，ここでは暗期だけに作用したと考えることができる．

中山[3,4]はアサガオの芽生で重金属酵素の阻害剤を暗期中に与えると花芽形成が抑制されることから，暗期反応は重金属酵素と密接な関係があると論じている．しかし，この場合，すでに述べたように

暗期に葉中に入った阻害剤が明期中も多少は葉に残っていたと考えられるので，必ずしも重金属酵素系が暗期反応のみと密接な関係を有するとは断言できない．ここに報告した実験では，KCN，NaN_3 ともに暗期中に与えるよりも明期中に与えた方が胞子果形成反応を強く抑制し，明期中に与えたこれら阻害剤の日長効果抑制作用はブドウ糖を添加することによって完全に消却された．しかも阻害剤と同時にブドウ糖を与えた場合には対照に比して日長効果が強められる．これらのことから考えると，重金属酵素阻害剤は明期中の光合成阻害を通じて日長効果を弱めるもので，炭水化物の十分な供給さえあれば逆に日長効果を強めるものと考えられる．

暗期にのみ重金属酵素阻害剤を与えた場合 KCN，NaN_3 では日長効果を弱め，CO では逆に日長効果を促進する．恐らく前二者の場合は明期中にも阻害剤が葉に残っており，そのために光合成が阻害されて日長効果も弱められたのに反し，後者の場合には暗期の重金属酵素系阻害の結果として日長効果促進作用のみが現われたのであろう．

ここに報告した実験ではマロン酸を与えることによって日長効果が著しく強められた．しかし，中山[3,4]はアサガオの子葉を暗期中マロン酸溶液に浸しても花芽形成は阻害されなかったと報告し，アサガオの芽生の花芽形成にはクエン酸回路は関与しないのではないかと考えた．一方，Liverman ら[6]はオナモミでクエン酸回路内の酸が明期反応と置きかわりうることを示し，花芽形成もこれらの酸で促進されると報告している．筆者も前報[7]においてそれらの酸のあるものが，サンショウモの胞子果形成を促進することを示した．ここに報告したマロン酸の胞子果形成促進作用は，明暗期いずれの反応に関与したものかわからないが，サンショウモではクエン酸回路を遮断することによって日長反応が強められたものと考えられる．ただし最近マロン酸を代謝しうる酵素系が植物で発見されている[8]ので，マロン酸がクエン酸回路遮断のみでなく，他の反応系にも作用して胞子果形成を促進したということも考えられ，この点さらに詳細な実験を必要とする．

As 塩または NaF を与えると，胞子果形成の阻害度に比してその発育が著しく不良であつたことから，分化した胞子果原基の発育には多量のエネルギーを必要とするものと考えられる．殊に As 塩を与えた場合単独では胞子果の発育が非常に悪かったのに対し，P 塩を同時に与えると正常になったことはこの推察を支持するものであろう．NaF も胞子果原基の分化に対する抑制はごく弱く，主として分化後の胞子果の発育を阻害するもののようである．

胞子果の発育阻害は KCN，NaN_3 を与えたときにもみられたが，これは恐らく光合成，またはこれによって生成された物質の有気的代謝の阻害を通じて起ったものと思われる．

この研究を行なうについては終始御援助をいただいた中山包教授に謝意を表する．

文　献

1) Kraus, F.J., and Kraybill, H.R., Ore. Agr. Exp. Sta. Bull. 149 (1918). 2) Merchers, G., und Glaes, H., Naturwiss. **31**: 249 (1943). 3) Nakayama, S., Bot. Mag. Tokyo, **68**: 61 (1955). 4) ——, Memo. Fac. Lib. Arts Edu. Miyazaki Univ. **1**: 7 (1957). 5) Shibata, O., Jour. Fac. Libe. Arts Sci. Shinshu Univ., 8 (Part 2): 7 (1958). 6) Liverman, J.L., and Bonner, J., Bot, Gaz. **151**: 121 (1953). 7) 柴田 治, 植雑, **72**: 462 (1959). 8) Giovanelli, J., and Stumpf, P.K., Plant Physiol. **32**: 492 (1957).

Summary

1) The experiments were undertaken to study the influences of certain enzyme inhibitors on the photoperiodic responses of a water fern, *Salvinia natans*.

2) Plants were subjected to 8-hour photoperiod for 5 days. KH_2AsO_4, NaF and malonic acid were added to the nutrient solution throughout the light and the dark periods. KCN and NaN_3 were applied during the light or the dark period, and CO only during the dark period.

3) KH_2AsO_4 and NaF inhibited the development of sporocarp in lower concentrations,

and the initiation of sporocarp in higher concentrations. The inhibitory effect of KH_2AsO_4 was removed by the simultaneous application of KH_2PO_4.

4) KCN and NaN_3, either being an aerobic inhibitor, inhibited sporocarp initiation especially when given to the plant during the light period. These enzyme inhibitors, however, promoted photoperiodic responses when supplied together with glucose.

5) CO and malonic acid considerably promoted sporocarp initiation.

6) It is considered that the interception of citric acid cycle promotes photoperiodic response, and that the inhibitors of heavy metal-containing enzyme inhibit photoperiodic response by preventing photosynthesis or related processes, but promote it if the sufficient carbohydrates are given to the plant.

本　会　記　事

昭和 34 年度会計決算報告 (昭和 34 年1月1日—昭和 34 年 12 月31 日)

収　入　の　部		支　出　の　部	
会　費	786,878	出　版　費	1216,864
予約購読料	346,898	発　送　費	152,084
一　部　売	20,150	編集関係費	114,300
バックナンバー	152,827	図書関係費	61,740
別刷代(立替分)	179,066	庶務関係費	260,145
文部省刊行補助	200,000	大会関係費	50,100
利　子	16,250	支　部　補　助	26,000
広　告　料	9,000	幹　事　手　当	162,000
小　計	1711,069	小　計	2043,233
前年度繰越金	720,420	次年度繰越金	388,256
総　計	2431,489	総　計	2431,489

Über die Wirkung der Pflanzendiffusate auf die Streckung des Sprosses und die Blütenbildung von *Pharbitis Nil* Chois.

von Yukiyoshi OGAWA* und Shun-ichiro IMAMURA*

Eingegangen am 9, September 1959

Seit Lang[1]) sind viele Fälle bekannt geworden, in denen Gibberellin die Blütenbildung der Pflanzen verschiedener photoperiodischer Typen fördert[2-7]). Es ist von Phinney[8]) und anderen Forschern[9-12]) gelungen, das Vorkommen von Gibberellin ähnlichen Substanzen in höheren Pflanzen nachzuweisen, Substanzen, die nicht nur auf das Streckungswachstum sondern auch auf die Blütenbildung regulierend zu wirken scheinen[13,14]). *Hyoscyamus niger* und *Samolus parviflorus* konnten durch Endospermsaft von *Echinocystis macrocarpa* unter nicht zusagenden Temperatur- und Lichtbedingungen zur Blütenbildung veranlasst werden[14]). Bei einer Kurztagpflanze, *Pharbitis Nil*, war dies auch der Fall, wie früher vorläufigerweise mitgeteilt worden ist[15,16]).

In der vorliegenden Arbeit werden weitere Beobachtungen über die Wirkung und Verbreitung der blühfördernden Substanzen in verschiedenen Pflanzenmaterialien mitgeteilt.

Material und Methode

Als Versuchspflanze dienten Keimlinge von ,,Kidachi'', einem Zwergmutanten von *Pharbitis Nil* Chois., der in unserem Laboratorium als Testpflanze für blühbeeinflussende Wirkung von aussen zugeführter Substanzen benutzt wird. Die Testmethode von Gibberellin mit ,,Kidachi'' ist an anderen Orten eingehend mitgeteilt worden[17]). ,,Kidachi'' ist nicht nur gegen blühfördernde sondern auch gegen streckungsfördernde Wirkung von Gibberellin sehr empfindlich. Man kann deshalb die beiden Wirkungen der Diffusate an ein und demselben Individuum beobachten.

Die nach H_2SO_4-Behandlung gründlich gewässerten Samen wurden in Saatkisten in 4 oder 5 Reihen ausgesät. Am zweiten und dritten Tage nach dem Auskeimen wurde der Vegetationspunkt mit Hilfe einer Mikropipette mit der Testlösung tropfenweise versetzt. Die Kontrollpflanzen, die in derselben Reihe mit den Versuchspflanzen standen, wurden mit Wassertropfen versetzt.

Nach Zufuhr von Diffusat wurden die Keimlinge Dunkelperioden verschiedener Dauer ausgesetzt, indem man jede Pflanzenreihe mit einer schmalen Kiste von geeigneter Grösse lichtdicht bedeckte. Eine besondere Vorrichtung zur Durchlüftung wurde dabei nicht angebracht.

Die Dauer der Verdunkelung, der die einzelnen Pflanzenreihen ausgesetzt wurden, war so gewählt, dass die Kontrollpflanzen bei längerer Dunkelperiode viele, dagegen bei kürzerer Periode keine Blütenanlagen bildeten. Meistens benutzten wir Dunkelperioden zwischen 8 und 12 Stunden mit Zeitintervallen von 1 oder 2 Stunden. Die kritische Dunkelperiode der Rasse liegt zwischen 8 und 9 Stunden; sie unterliegt einer Schwankung je nach den inneren und äusseren Bedingungen. Selbst unter konstanten Aussenbedingungen lässt sich eine Änderung in der photoperiodichen Empfindlichkeit

* Laboratorium der Angewandten Botanik der Landwirtschaftlichen Fakultät, Kyoto Universität, Kyoto, Japan.

beobachten. Bei gedämpfter Empfindlichkeit kann oft eine 10-stündige Dunkelperiode unwirksam sein. Daher ist es ratsam, mit Dunkelperioden von verschiedener Dauer zu arbeiten.

Nach experimenteller Behandlung, bei der die Häufigkeit von Stoffzufuhr und Verdunkelungsdauer bei einzelnen Versuchen variierten, wurden die Pflanzen in Gewächshaus unter ununterbrochener Beleuchtung kultiviert mit näturlichem Licht am Tage und Glühlampen in der Nacht.

Etwa drei oder vier Wochen nach der Dunkelbehandlung, d. h. etwa 4 oder 5 Wochen nach dem Aussäen, wurde die Sprosslänge oberhalb der Kotyledonen gemessen und mit einem Binokularmikroskop bei schwacher Vergrösserung auf Blütenanlagen untersucht. Als Massstab der Blühreaktion dienten die Zahl der Pflanzen mit den Blühtenanlagen (Blühprozent) und die durchschnittliche Zahl der Blühtenanlagen pro Pflanze. Bei Versuchen mit schwacher Induktion, wie es in der vorliegenden Untersuchung der Fall ist, laufen beiderlei Grössen parallel.

Wie schon früher berichtet, unterliegt die Empfindlichkeit dieser Pflanze aus einer noch unbekannten Ursache einer ziemlich starken Schwankung; nur die Daten von ein und demselben Versuche, die am bestimmten Tage unter bestimmten Bedingungen erhalten wurden, sind miteinander vergleichbar.

Versuchsergebnisse

A. Wirkung der Diffusate von unreifen Samen von *Pharbitis Nil* und *Phaseolus vulgaris.*

Wie aus Tabelle 1 zu ersehen ist, ruft das Diffusat von *Pharbitis* eine deutliche Förderung der Sprossachsenstreckung sowie der Blütenanlagebildung hervor. Mit abnehmender Dauer der Dunkelperiode tritt aber die Blühförderung immer schwächer zutage. Bisher gelang es uns nicht, die Blütenbildung bei einer Dunkelperiode von weniger als 7 Stunden hervorzurufen. Da die kritische Dunkelperiode der benutzten Rasse zwischen 8 und 9 Stunden liegt, kann man sehen, dass die Blühförderung sich nur bei kritischer Dunkelperiode beobachten lässt. Das Diffusat von *Phaseolus vulgaris* hat auch eine ähnliche Wirkung (Tabelle 2).

B. Vorkommen der wirksamen Substanzen in verschiedenen pflanzlichen Materialien.

Um die Verbreitung der wirksamen Substanzen im Pflanzenreich zu erforschen, wurden Extrakte aus vielen Organen von verschiedenen Familien angehörigen Pflanzen auf ihre Wirkung geprüft. Stets wurden junge Organe zur Untersuchung herangezogen. Die Materialien wurden, wenn nötig, mit Messer geschnitten und meistens nach Phinney[8]) in wasserhaltiges Azeton hineingetan. In einigen Versuchen wurden sie an Tierkohle adsorbiert und mit Azeton eluiert, um Begleitstoffe zu entfernen.

Die Wirkung wurde nach dem Augenmass geschätzt und in einigen Fällen mit der von Gibberellin verglichen, das eine ähnliche Wirkung auf diese Pflanze ausübt. Oft erfährt der Vegetationspunkt durch das Diffusat eine mehr oder minder starke Schädigung. Auch ist das Herausdiffundieren der Substanz aus den Materialien nicht immer vollständig und kann in einzelnen Fällen verschieden stark sein. Daher können die Ergebnisse zwar nicht eine hohe Genauigkeit in Anspruch nehmen, doch können sie eine ungefähre Kenntnis über die Verbreitung der Substanzen in verschiedenen Pflanzenmaterialien geben (Tabelle 3).

Besonders wirksam waren die Samen von *Pharbitis Nil*, *Quamoclit angulata*,

Tabelle 1. Einfluss des Diffusats von *Pharbitis*-Samen auf die Blütenbildung von *Pharbitis Nil.*

Versuchsnummer und Datum	Dunkelperiode in Stunden	Mit Diffusate behandelt				Kontrolle				Längenverhältniss d. Stengels (A/B)
		Zahl d. Versuchspflanzen	Blühprozent	Blütenzahl pro 10 Pflanzen	Länge d. Stengels (A)	Zahl d. Versuchspflanzen	Blühprozent	Blütenzahl pro 10 Pflanzen	Länge d. Stengels (B)	
Versuch 1 Ausgesät: 19, Sept., mit Diffusat behandelt: 21 und 22, Sept., Dunkelbehandlung: 24, Sept. 1958.	12	7	100	22.9	25.6	8	12	2.5	19.0	1.20
	11	11	100	18.2	25.5	11	0	0	19.8	1.28
	10	9	89	10.0	27.7	11	0	0	19.8	1.38
	9	7	14	1.4	23.7	7	0	0	19.9	1.19
	8	12	0	0	25.0	9	0	0	19.3	1.29
Versuch 2 Ausgesät: 2, Okt., mit Diffusat behandelt: 3 und 4, Okt., Dunkelbehandlung: 4, Okt. 1958.	11	27	100	25.9	33.9	30	0	0	28.5	1.18
	10	29	90	19.7	32.1	33	0	0	29.9	1.07
	9	32	34	5.0	35.1	23	0	0	29.5	1.18
	8	30	7	0.7	33.2	28	0	0	29.1	1.14
Versuch 3 Ausgesät: 4, Okt., mit Diffusat behandelt: 7 und 8, Okt., Dunkelbehandlung: 10, Okt. 1958.	11	17	88	22.9	33.6	15	7	0.7	20.5	1.63
	10	16	69	18.1	38.4	12	0	0	23.2	1.65
	9	15	7	0.7	34.9	14	0	0	21.1	1.65
	8	17	6	0.6	36.2	14	0	0	23.4	1.86
	7	16	0	0	28.5	15	0	0	17.5	1.62
Versuch 4 Ausgesät: 8, Okt., mit Diffusat behandelt: 11, 12 und 13, Okt., Dunkelhandlung: 14, Okt. 1958.	12	16	100	41.9	54.4	14	71	8.6	31.9	1.70
	10	12	75	16.3	59.9	11	0	0	32.8	1.64
	8	16	6	0.6	55.1	13	0	0	36.5	1.50
	6	17	0	0	51.2	15	0	0	29.7	1.72
	4	15	0	0	54.3	13	0	0	30.9	1.75

Tabelle 2. Einfluss des Diffusats von *Phaseolus*-Samen auf die Blütenbildung von *Pharbitis Nil*.

Versuchsnummer und Datum	Dunkelperiode in Stunden	Mit Diffusat behandelt				Kontrolle				Längenverhältniss d. Stengels (A/B)
		Zahl d. Versuchspflanzen	Blühprozent	Blütenzahl pro 10 Pflanzen	Länge d. Stengels (A)	Zahl d. Versuchspflanzen	Blühprozent	Blütenzahl pro 10 Pflanzen	Länge d. Stengels (B)	
Versuch 1 Ausgesät: 26, Sept., mit Diffusat behandelt: 28 und 29, Sept., Dunkelbehandlung: 30, Sept., 1958.	12	23	100	39.6	46.6	21	72	14.3	24.9	1.87
	11	24	100	40.8	45.0	18	50	6.7	25.8	1.74
	10	23	52	11.7	46.3	21	0.5	0.5	27.1	1.69
	9	21	33	5.6	42.0	22	0	0	27.0	1.55
	8	15	13	1.3	42.0	13	0	0	26.0	1.50
Versuch 2 Ausgesät: 1, Okt., mit Diffusat behandelt: 3 und 4, Okt., Dunkelbehandlung : 5, Okt. 1958.	11	18	95	20.0	30.0	22	0	0	27.4	1.10
	10	20	60	10.0	31.8	23	0	0	25.3	1.25
	9	20	41	7.3	26.6	22	0	0	24.2	1.20
	8	22	5	0.4	29.0	22	0	0	24.2	1.20
	7	20	0	0	31.6	20	0	0	24.1	1.32

Tabelle 3. Vorkommen der wirksamen Substanz in verschiedenen Pflanzen.
Etwa 100 g. Material extrahiert und in etwa 1 ml. Wasser aufgenommen.
Az: Extraktion mit wasserhaltigem Azeton; Al mit Alkohol; Ä: mit Äther und W: mit Wasser. T: adsorbiert mit Tierkohle aus wässeriger Lösung und eluiert mit Azeton. S: behandelt direkt mit Samensaft. Wirkung ± sehr schwach, + schwach, ++ deutlich, +++ stark, ++++ sehr stark, ++++ entspricht ungefähr der Wirkung von 50–100 μg./ml. Gibberellin-Lösung.
Ausgeführt von Mai bis November 1958.

Pflanze	Organ	Wirkung auf		Bemerkung
		Sprossstreckung	Blütenbildung	
Gingko biloba	Endosperm	–	–	Az
	〃	–	–	Az, T
Coix Ma-Yuen	Frucht	±	–	Az
	〃	–	+	Ä
Oryza sativa	Frucht	+	–	Az
Zea Mays	Frucht	±	–	Az
	〃	+	–	Az, T
	〃	+	–	W, T
	Kolben	–	–	Az
Trachycarpus excelsa	Blütenstand	–	–	Az
	〃	–	–	Az, T
	〃	–	–	W, T
Ficus Carica	Blütenstand	–	–	Az
	〃	–	–	Az, T
Celosia cristata	Blütenstand	–	–	W
Raphanus sativus	Blütenstand	+	–	Az
	〃	+	–	Az, T
	〃	+	–	W, T
Ricinus communis	Frucht	–	–	Az
Hibiscus Manihot	Frucht	–	–	Az
Arachis hypogaea	Samen	++	–	Az, T
	Gynostemium	–	–	Az
Canavallia ensiformis	Samen	++	++	Az
Cassia occidentalis	Samen	+	–	Az
	Hülsenwand	–	–	Az
Glycine Soja	Samen	±	–	Az
	〃	±	–	Az, T
	Hülsenwand	–	–	Az
Lupinus luteus	Samen	++++	+++	Az
	〃	++++	++	Az, T
	Hülsenwand	++	+++	Az

Tabelle 3. (Fortsetzung)

Pflanze	Organ	Wirkung auf		Bemerkung
		Sprossstreckung	Blütenbildung	
Phaseolus angularis	Samen	±	−	Az
	Hülsenwand	±	−	Az
Phaseolus vulgaris	Samen	+++	+++	Az
	〃	+++	++	Az, T
	Hülsenwand	+	+	Az
Vicia Faba	Samen	±	−	Az
	〃	±	−	Az, T
	Hülsenwand	−	−	Az
Vigna Catiang var. *sinensis*	Samen	±	−	Az
Vigna sinensis	Samen	+	−	Az
	〃	+	−	Az, T
	Blütenstiel	−	−	Az
Calonyction bona-nox	Samen	+++	+++	Az
	Blütenstiel	−	−	Az
Pharbitis Nil	Spross im Langtag	−	−	Az
	〃	±	−	Az, T
	Spross im Kurztag	−	−	Az
	〃	±	−	Az, T
	Etiolierte Keimlinge	±	−	Az
	〃	±	−	Az, T
	Samen	+++	+++	Az
	〃	+++	+++	W
	〃	+++	+++	Al
	〃	++	++	Ä
	〃	++	++	S
Quamoclit angulata	Samen	++	++	Az
Quamoclit pennata	Samen	++	++	Az
Sesamum indicum	Frucht	−	−	Az
Sechium edule	Samen	++++	+++	Az
	〃	++++	+++	Al
	〃	++++	+++	S
Cosmos bipinnatus	Blütenstand	−	−	Az
Erigeron annuus	Blütenstand	±	−	Az
Helianthus annuus	Blütenstand	−	−	Az
Helianthus tuberosus	Knollen	−	−	Az
Rudbeckia bicolor	Blütenstand	±	−	Az
Xanthium canadense	Frucht	−	−	Az

Quamoclit pennata, Calonyction bona-nox (Convolvulaceae), *Sechium edule* (Cucurbitaceae), *Canavallia ensiformis, Phaseolus vulgaris, Lupinus luteus* (Leguminosae). Ein grosser Unterschied im Gehalt lässt sich aber zwischen nahe verwandten Pflanzen wie *Phaseolus vulgaris* und *Phaseolus angularis* beobachten. Auch haben die Hülsenwände der genannten Leguminosen und die Frucht von *Coix Ma-Yuen* eine schwache blühfördernde Wirkung gezeigt. Mit wenigen Ausnahmen geht die Wirkung der Diffusate auf die Blütenbildung mit der Streckungsförderung der Sprossachse Hand in Hand. In den meisten Fällen aber tritt die erstere viel schwächer auf als die letztere: Viele Diffusate aus Samen und Blütenständen fördern zwar die Sprossstreckung, nicht aber die Blütenbildung. Bei *Pharbitis* zeigt das Diffusat aus etiolierten Keimlingen und Sprossen nur eine schwache Streckungs- aber keine Blühförderung. Die photoperiodischen Bedingungen, unter deren *Pharbitis* gezogen war, üben keinen Einfluss auf die Wirksamkeit der Diffusate aus Sprossen.

Die Substanzen können mit Wasser, Äthylalkohol und Äther ebenso gut wie mit wasserhaltigem Azeton ausgelaugt werden, und lassen sich aus wässeriger Lösung durch Tierkohle adsorbieren.

Schlussbetrachtung

Dass Gibberellin Blütenbildung und Streckung des Blütenschafts hervorrufen kann, ist eine bekannte Tatsache. Gibberellin kann nicht nur die Kälte bei vielen kältebedürftigen Pflanzen sondern auch den Langtag bei vielen Langtagpflanzen ersetzen. Nachdem über eine hemmende Wirkung bei den meisten untersuchten Kurztagpflanzen mitgeteilt wurde[2,18]), sind zwei Fälle bekannt geworden, wo Gibberellin auch eine blühfördernde Wirkung auf die Ausbildung von Blütenanlagen bei den Kurztagpflanzen zeigte[15,19]). *Xanthium saccharatum* und *Pharbitis Nil* können durch Zufuhr von Gibberellin-Lösung mehr Blütenanlagen, im Vergleich mit den Kontrollpflanzen, ausbilden. Aus der Arbeit von MacMillan[20]), der Gibberellin A_1 aus unreifen Samen von *Phaseolus multiflorus* isoliert hat, scheint die blühfördernde Wirkung der pflanzlichen Diffusate auf Gibberellin zurückzugehen. Da bei den in der vorliegenden Arbeit beschriebenen Versuchen die Blühförderung mit der Förderung der Streckung der Sprossachse Hand in Hand geht, können wir auch hier mit etwas ähnlichem zu tun haben. Bisher gelang es uns nicht, die Blütenbildung von *Pharbitis* bei ununterbrochener Beleuchtung hevorzubringen. Wie von Okuda eingehend berichtet wurde[21]), ruft Gibberellin eine bedeutende Förderung der Zellteilung in jungen Geweben hervor und beschleunigt das Auftreten von Blattanlagen am Vegetationspunkt. Dies ist auch der Fall bei den wirksamen Pflanzendiffusaten. Es ist höchst wahrscheinlich, dass der wirksame Stoff durch Steigerung der Aktivität des Vegetationspunktes die Wirksamkeit des in der Dunkelperiode im Blatt ausgeübten Effektes verstärkt und dadurch indirekt die Blütenbildung fördert.

Doch beobachteten wir in einigen Fällen, dass die Blühförderung keine Steigerung der Sprossstreckung mit sich führt. Ob wir durch irgend eine chemische Behandlung der Diffusate oder sonstwie die beiden Wirkungen trennen können oder durch Zugabe grösserer Mengen der Substanz die Blütenbildung unter kontinuierlicher Beleuchtung hervorrufen können, steht noch offen.

Zusammenfassung

1) Das Diffusat von unreifen Samen von *Pharbitis Nil* fördert die Streckung der Sprossachse und die Blütenbildung bei einem Zwergmutanten derselben Art unter kritischen photoperiodichen Bedingungen.

2) Ähnliche Substanzen sind weit verbreitet und kommen in grossen Mengen vor besonders in den Familien der Convolvulaceae, Cucurbitaceae und Leguminosae.

3) Die blühfördernde Wirkung kann zur Zeit von der streckungsfördernden Wirkung nicht getrennt werden. Ebensowenig konnten die Pflanzen durch die geprüften Substanzen unter ununterbrochener Beleuchtung zur Blütenbildung veranlasst werden.

Literaturverzeichnis

1) Lang, A., Naturwiss. **43**: 284 (1956). 2) Lona, F., L'Ateneo parmense **27**: 867 (1956). 3) Bünsow, R., und Harder, R., Naturwiss. **43**: 479 (1956). 4) Bünsow, R., und Harder, R., Naturwiss. **43**: 527 (1956). 5) Lang, A., Proc. Nat. Acd. Sci. **43**: 709 (1957). 6) Witiwer, S. H., and Bukovac, B. J., Quart. Bull. Michigan Agri. Exp. Stat. **39**: 661 (1957). 7) Carr, D. C., McComb, A. T., and Osborn, L. D., Naturwiss. **44**: 428 (1957). 8) Phinney, B. O., West, C. A., Pitzel, M., and Neelz, P. M., Proc. Nat. Acd. Sci. **43**: 398 (1957). 9) Mitchell, J. W., Skaggs, D. P., and Anderson, W. P., Science **114**: 159 (1951). 10) Radly, M., Nature **178**: 1070 (1956). 11) McComb, A. J., and Carr, D. T., Nature, **181**: 1548 (1958). 12) Murakami, Y., Bot. Mag. Tokyo **72**: 36 (1958). 13) Bünsow, R., Penner, J., und Harder, R., Naturwiss. **45**: 46 (1958). 14) Lang, A., Sandval, A. J., and Besri, A., Proc. Nat. Acd. Sci. **43**: 960 (1957). 15) Ogawa, Y., und Imamura, S., Proc. Japan Acad. **34**: 629 (1958). 16) Ogawa, Y., und Imamura, S., Proc. Japan Acad., **34**: 631 (1958). 17) Hirono, Y., Ogawa, Y., und Imamura, S., Plant and Cell Physiol. **1**: 81 (1960). 18) Harder, R., und Bünsow, R., Planta **51**: 201 (1958). 19) Lincoln, R. G., and Hamner, K. C., Plant Physiol. **33**: 101 (1958). 20) McMillan, J., und Suter, P. J., Naturwiss. **45**: 46 (1958). 21) Okuda, M., Bot. Mag. Tokyo **72**: 443 (1959).

摘　　要

アサガオの茎の伸長および花芽形成に対する植物浸出物の作用について

小川幸持・今村駿一郎

1. アサガオの未熟種子からの浸出物は限界日長条件で矮性朝顔の茎の伸長および花芽形成を促進する.

2. 同じような物質は他の植物にも広く分布し，特にヒルガオ科，ウリ科，マメ科の植物に多い.

3. この花芽形成促進作用は今までのところ茎の伸長促進作用と分離することができない. またこの物質によって連続光の下で花芽形成を起させ得ない.（京都大学農学部応用植物学研究室）

Ecological and Physiological Studies on the Vegetation of Mt. Shimagare
IV. Some Physiological Functions Concerning Matter Production in Young *Abies* Trees*

by Sumio KUROIWA**

Received September 2, 1959

It is not only physiologically but also ecologically of interest how and to what extent the physiological function of a plant is affected by its life history. Concerning this problem, many workers[1-14] have studied especially the shade tolerance of plants, and distinguished the differentiation in photosynthetic response to light of the sun- and the shade-leaves. Ecologically to make clear the development of a plant community, however, an important problem firstly to be solved is whether such a differentiation exists between the size classes of constituent plants. According to a previous paper[15], the light factor in the *Abies* stand on Mt. Shimagare is microclimatically extremely different among tree classes, as the canopy of each class is situated at different strata where the illumination rapidly decreases towards the ground.

In the present paper, the dominant, intermediate and suppressed trees in a 20-year-old *Abies* stand of Forest Unit V are compared with each other in photosynthesis, respiration, and nitrogen content of organs. These characteristics will also be compared between *Abies Veitchii* and *A. Mariesii*, which are two main species of the subalpine coniferous forest. Here will be discussed the age effect of needles on their photosynthesis and respiration[16-21], and the relation of branch and trunk diameter to their respiration as studied in beech by Möller *et al.*[22].

Method

From each of the dominant, intermediate and suppressed classes which were classified on the basis of the frequency distribution of tree height[15], a few samples of *Abies* were selected, being respectively about 110, 80 and 60 cm. in average height and about 230, 60 and 8 g. in average dry weight. Photosynthesis (at 0.03 vol. per cent of CO_2) and respiration were measured by the partially improved Boysen Jensen method[3,23] mostly in the summer seasons of 1957 and 1958. In one-year-old needles (current) and branchlets, however, the measurements were performed late in August after their maturity. Nitrogen content of needles and branchlets was determined by the micro-Kjeldahl method.

The intact or the detached branchlets covered with needles of the same age, being selected from a shoot of a big lively branch, were used for the measurements of photosynthesis at the laboratory under controlled conditions of illumination and temperature. Sometimes, photosynthesis measurements were also made in intact shoots under field conditions. The photosynthesis in needles themselves was assessed by adding the respiration of the branchlet without needles to the amount of photosynthesis of the shoot as a whole. Light intensity was determined just above the

* Supported by the Grant in Aid of Scientific Research of the Ministry of Education.
** Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

assimilation chamber with the electric photometer (Toshiba No. 5), and leaf temperature, by means of the copper-constantan thermoelement (dia.: 0.02 mm.) at the lower side of needle in the assimilation chamber. In the field the leaf temperature was deduced from the air temperature in the assimilation chamber by using the difference between both temperatures determined in the laboratory.

Respiration was measured in various organs; needles of each age, cylindrical branchlets which were divided into each branchlet diameter class and into each age class, an entire conic trunk and a whole root system. These organs were put into a dark respiration chamber under moist condition, shortly after being detached from a tree. For example, the entire root system was measured in respiration immediately after it was cut off from the stem, entirely washed, and wiped with filter paper.

Photosynthesis

Net photosynthesis in a shoot depends not only on the photosynthesis in the leaves but also on the respiration in the leaves and the branchlets. In relation to temperature, this fact must induce that the optimum temperature for net photosynthesis of shoot decreases with diminishing illumination intensity as shown by Pisek and Winkler[24]). However, the gross photosynthesis computed from their data has shown a constant optimum temperature independent of illumination. Fig. 1 indicates gross photosynthesis curves of *A. Veitchii* illustrated against leaf temperature; they were observed under a saturated illumination of 30 kilolux in the needles of various years old. The optimum leaf temperature was found at about 20° independent of needle ages. Nearly the same optimum temperature was obtained for needles of 1 and 3 years old of *A. Mariesii*, under the same light condition. As a result of these investigations it seems generally to be concluded that the optimum leaf temperature for gross photosynthesis in *Abies* needles is 20° independently of illumination intensity and needle age. So the light-photosynthesis curve was determined at the temperature. The photosynthetic rate obtained under the field conditions varying in illumination and temperature was revised to the value at a leaf temperature of 20° in the corresponding illumination, according to the relation of photosynthesis to leaf temperatures shown in Fig. 1.

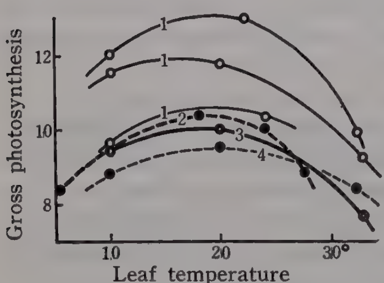


Fig. 1. Gross photosynthesis (mg. CO_2/100 sq. cm. of unilateral needle area/hr.) at 30 kilolux for the intermediate *Abies Veitchii* tree of 20 years old. Figures on the curves stand for needle age. Three curves for one-year-old needles, from lower to upper, correspond to photosynthesis in very young (in June), just matured (in July) and matured needles (in August), respectively. Mean values for a few measurements.

The light-gross photosynthesis curves (on a dry weight basis) for new needles of *A. Veitchii* are given in Fig. 2 which indicates the difference between the dominant and intermediate: the former was possessed of a higher photosynthetic rate of needles than the latter's, over the whole range of light intensity adopted. In each of needles of various years old the light-saturated photosynthetic rate decreased with the diminution in tree size (Table 1). Such differences among tree classes became more decisive on a needle area basis than on a needle weight basis (Fig. 2), because of the large thickness of needles in the dominant (Fig. 3). For instance, in the needles of 2 years old of *A. Mariesii*, the light-saturated gross photosynthetic rates for the

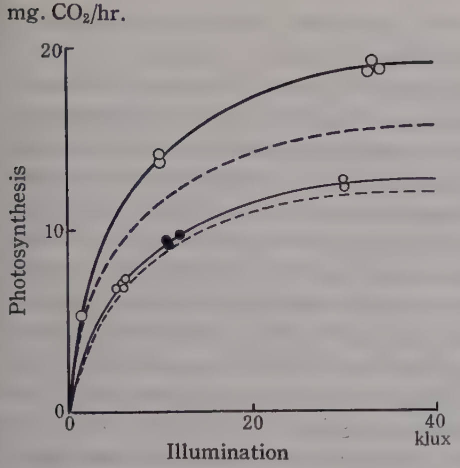


Fig. 2. Light-photosynthesis curves of one-year-old needles in *Abies Veitchii* trees 20 years old, at 20°. Upper two curves, dominant tree; lower two curves, intermediate tree. Solid lines, on a needle area basis (100 sq. cm., unilateral); broken lines, on a needle dry weight basis (g.). Open circles, at the laboratory; solid circles, in the field.

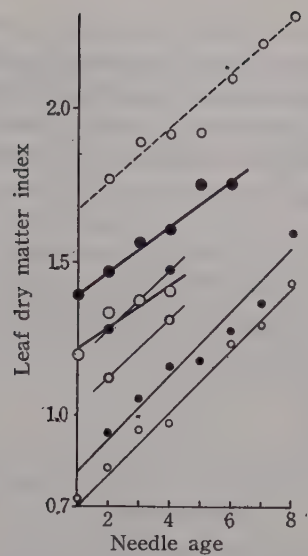


Fig. 3. Relation between needle age (year) and "leaf dry matter index" (g. needle dry weight/100 sq. cm. of unilateral needle area) in *Abies Veitchii* (open circles) and *A. Mariesii* (solid circles), 20 years old. Large circles, dominant trees; small ones, suppressed trees; and inbetween, intermediate trees. Uppermost, broken line indicates a intermediate *A. Veitchii* tree of 40 years old.

Table 1. Net photosynthesis, respiration (at 20°) and nitrogen content in needles of the dominant, intermediate and suppressed trees of 20 years old of *Abies Veitchii* (*A. V.*) and *A. Mariesii* (*A. M.*). The values in parentheses were estimated from the relationship between physiological activity and nitrogen content (Fig. 9).

Tree class	Needle age	Photosynthesis at light saturation				Compensation point		Respiration		Nitrogen content	
		mg. CO_2/g.d.w., hr.		Illumination kilolux		lux		mg. CO_2/g.d.w., hr.		mg. N/g.d.w.	
		A. V.	*A. M.*	*A. V.*	*A. M.*	*A. V.*	*A. M.*	*A. V.*	*A. M.*	*A. V.*	*A. M.*
Dominant	1	15.0	8.6	40	35	600	500	1.03	0.98	—	16.0
	2	—	7.3	—	30	—	500	0.92	0.85	16.7	14.0
	3	10.2	5.7	32	25	650	500	0.82	0.78	16.2	13.4
	4	—	—	—	—	—	—	0.71	0.55	15.1	13.2
	5	3.1	2.3	25	20	500	500	0.60	0.46	14.4	12.5
Intermediate	1	11.4	7.0	35	30	700	600	0.98	0.94	16.5	14.8
	2	8.4	—	30	—	600	—	0.86	0.77	16.0	13.5
	3	7.4	(5.0)	—	(15)	—	—	(0.78)	(0.63)	15.7	12.5
	4	6.7	—	—	—	—	—	0.60	—	14.8	11.9
Suppressed	1	—	—	—	—	—	—	0.94	—	16.2	13.0
	2	6.3	5.3	8	8	500	500	0.75	0.62	15.7	12.6
	3	—	(4.5)	—	(8)	—	—	—	(0.45)	14.6	12.2
	4	—	3.2	—	8	—	600	0.29	0.28	13.5	11.8

dominant and the suppressed were on a needle area basis in the ratio of 100:50, while on a needle dry weight basis they were 100:73.

With ageing of needles, the light-saturated photosynthesis in the intermediate *A. Veitchii* decreased over a whole temperature range (Fig. 1) and the photosynthesis measured in the dominant *A. Mariesii* at an optimum leaf temperature of 20° decreased over a whole illumination range (Fig. 4). The same tendency was also observed in each tree class of both tree species (cf. Tab. 1). Therefore, it is generally conclusive that with ageing of needles the photosynthetic rate decreases in every tree class over whole ranges of illumination and temperature. This depression in photosynthesis becomes more conspicuous on a dry weight basis than on a needle area basis, as the needles increase in their thickness with ages.

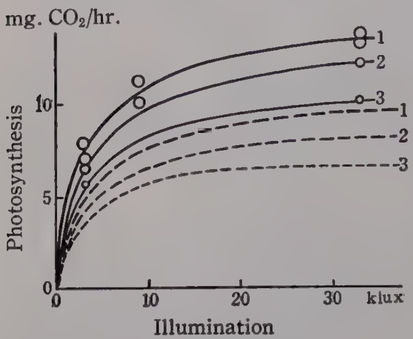


Fig. 4. Needle age effect on photosynthesis at 20°, in the dominant *Abies Mariesii* tree of 20 years old. Figures stand for needle age. Solid lines, on a needle area basis (100 sq. cm., unilateral); broken lines, on a needle dry weight basis (g.).

The light-saturation point in photosynthesis was generally higher in the dominant than in the suppressed, and further in the former the difference was observed among needles of different ages, but in the latter it was not found at all (Table 1). Such a difference between tree classes seems to be due to the difference in light environment between them. In the suppressed, all needles are shaded by the canopies of the larger tree classes. — Relative light intensities of only 6 and 4% in average were respectively obtained at the upper and lower parts of the canopies of suppressed trees in an *Abies* stand of 20 years old[15]).—So all of them were naturally characterized with shade-leaves of lower light-saturation point. In the dominant trees, illumination prevailing on the needles would decrease with ageing of needles from full light to depressed one, as the older needles distributed inside the crown were shaded by younger needles developing at the open outside of the crown. Consequently, the older the needles, the more distinctly they behave as shade-leaves in photosynthetic characteristics.

In each of needles of various years old of respective tree classes, the light-saturated photosynthesis and the light-saturation point were higher in *A. Veitchii* than in *A. Mariesii* (ref. Table 1). The difference in photosynthetic rate between these two species was shown more clearly on a needle weight basis than on a needle area basis, because the former was thinner in needle thickness than the latter.

The above-mentioned optimum leaf temperature for photosynthesis in these subalpine *Abies* species (the annual mean air temperature at Mt. Shimagare was assessed as −0.3°[25])) appears to be fairly lower than that in broad-leaved evergreen trees which constitute the laurel forest in the southern part of Japan. As to the latter, the optimum leaf temperature of about 30° in summer, and of about 25° in winter can be estimated from the air temperature optimal for photosynthesis which was determined by Kusumoto[20]) at Kagoshima where annual mean air temperature is 16.6°. These facts may suggest the general trend that colder the habitat the lower the optimum leaf temperature for photosynthesis, in accordance with Pisek and Winkler's[24]) where the temperature optimum for photosynthesis was discussed in *Picea excelsa* and *Pinus cembra* etc. Furthermore, an optimum temperature of about 18° was reported by

Lundegårdh[5]) for net photosynthesis at normal CO_2 concentration in major crop plants in Sweden. This optimum temperature, which seems rather too low, would be induced by the fact that his temperature-photosynthesis curve was made on the basis of the air temperature in the assimilation chamber instead of real leaf temperature which must be responsible for the physiological functions. Even though the assimilation chamber is sunk in cool running water, the leaf temperature should be generally higher than the air temperature on account of overheating of leaf by radiation, as reported by Tranquillini[27]). The leaf temperature which was measured in the assimilation chamber under the same conditions as in the photosynthetic measurement (0.006 m./sec. air movement; 30 kilolux light intensity; at 10 cm. depth in running water), was 2.7° at *Fagopyrum esculentum* leaves (cf. Fig. 5) and 2.0° at *Abies* needles higher than an air temperature of 20° in the chamber. Such a temperature difference increased at lower air temperature but decreased at higher one, as shown e.g. in Fig. 5. Provided that temperature-photosynthesis curve is configured, as seen e.g. in Walter[28]), Stålfelt[29]), Lundegårdh[5]), Kusumoto[26,30]), through plotting photosynthetic rates against air temperatures, the obtained curve will give an underestimated optimum temperature, as the photosynthetic rate will be overestimated in the lower temperature range but underestimated in the higher temperature range in comparison with the real photosynthetic rate against the leaf temperature.

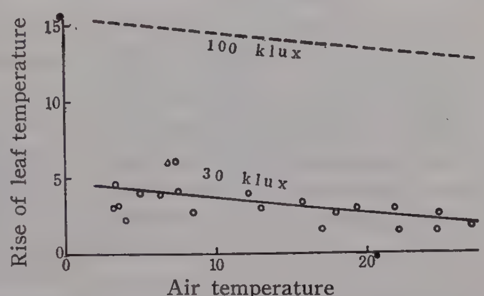


Fig. 5. Rise of leaf temperature of *Fagopyrum esculentum* compared with air temperature in the assimilation chamber.

Respiration

The dominant, intermediate and suppressed *Abies* were compared with each other in respiration of needles of the same age (cf. Table 1). The needle respiration, especially on a needle area basis, was the highest in the dominant and the lowest in the suppressed, as the needle thickness increased with increasing of tree size (see Fig. 3). In each of these tree classes, the needle respiration was decreased with ageing of needles, and it appeared more clearly on a needle weight basis than on a needle area basis, on account of an increase in needle thickness with needle ages. In each needle age and tree class, the needles of *A. Veitchii* had a somewhat higher respiration than that of *A. Mariesii* on a weight basis, but not always on an area basis. These relationships of the needle respiration to the tree class, needle age and tree species accord with those in photosynthetic activity already mentioned. This can be elucidated by a close relationship between respiration rate and photosynthetic rate in Fig. 6, where it is represented in a curvilinear line different from the linear one observed in rice plants[31,32]).

In the various tree classes of the *Abies* stands of 14, 20 and 23 years old, the respiration rates measured at 25° for entire conic trunk and cylindrical branchlet were plotted against the basal diameter of trunk and the branchlet diameter measured at the middle of branchlet (Fig. 7–A and –B). In each tree class, the respiration rate on a dry weight basis gradually decreased with increase in diameter throughout the branchlets and trunks (Fig. 7–A), while the respiration rate on a surface area basis

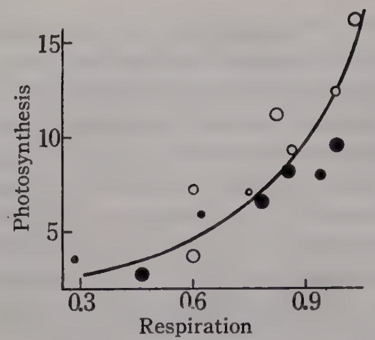


Fig. 6. Relation of respiration to gross photosynthesis light-saturated at 20°. Unit is mg. CO_2 /g. needle dry weight/hr. Large circles, dominant tree; middle circles, intermediate tree; small circles, suppressed tree. Open circles, *Abies Veitchii*; solid ones, *A. Mariesii*.

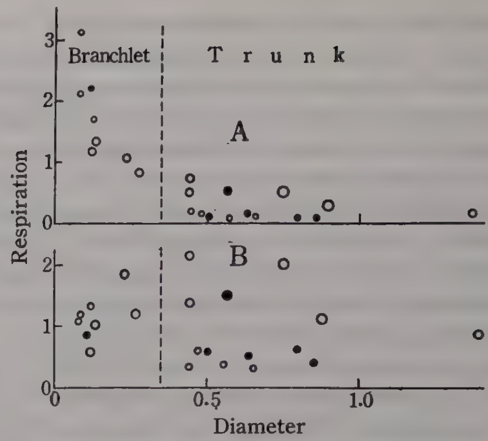


Fig. 7. Relation of respiration (mg. CO_2 output per hr., at 25°) of entire trunk to the basal diameter (cm.) and that of branchlet to its diameter (cm.). A, on g. dry weight basis; B, on 50 sq. cm. surface basis. 14-, 20- and 23-year-old *Abies* stands. Signs mean the same as in Fig. 6.

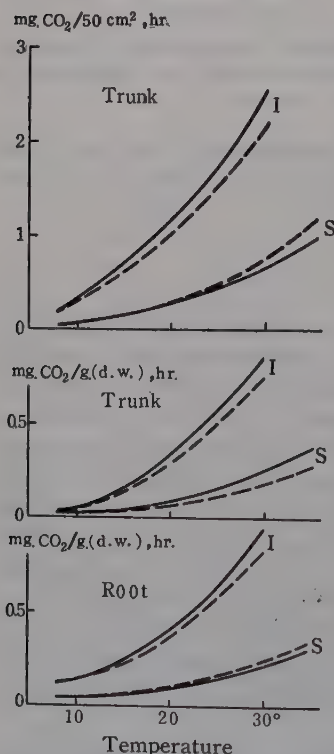


Fig. 8. Temperature-respiration curves for entire trunk and root system of *Abies* trees 20 years old. I, intermediate trees; S, suppressed trees. Solid lines, *A. Veitchii*; broken lines, *A. Mariesii*.

was related to diameter obscurely in the trunk but reversely in the branchlet (Fig. 7–B). These facts accord with the results obtained by Möller *et al.*[22]) in beech. The respiration rate of trunk with the same diameter was the lowest in the suppressed, but the respiration rate of the dominant, on a surface as well as on a weight basis, was not always higher than that of the intermediate. In Fig. 8, regarding temperature-respiration curves for trunk and root system the difference is found between the intermediate and the suppressed, whose basal diameters were 0.5 and 0.3 cm., respectively, and the curves obtained in the suppressed are much lower than those in the intermediate over the whole range of measured temperature. Between both *Abies* species, there was no much difference in the respiration rates of trunk, branchlet and root system (see Figs. 7 and 8).

Nitrogen content

It has been revealed by many authors[17,38–42]) that deficiencies in mineral nutrients depress photosynthetic activity as well as plant growth, and by some of them[38,39,41,42]) that there exists a close relationship between photosynthetic activity and nitrogen content of leaves. In this connection some investigations were carried out in needles and branchlets of the *Abies*.

Tree classes were compared with each other in the total nitrogen content in each of needles of various

years old (cf. the last column of Table 1). The content decreased on a dry weight basis with aging of needles, but not always on a needle area basis because of large thickness of aged needles. In the needles of the same age, the nitrogen content decreased in the trees of the lower size class, and the decrease was expressed more markedly on an area basis than on a weight basis, as the needle thickness diminished with tree size diminution. For instance, the nitrogen content in the two-year-old needles of dominant, intermediate and suppressed *A. Mariesii* was on an area basis in the ratio of 100:84:57, and on a dry weight basis 100:96:90. The needle nitrogen content was higher in *A. Veitchii* than in *A. Mariesii* on a dry weight basis, but almost the same in both *Abies* species on a needle area basis, on account of the larger thickness of the *Mariesii* needles.

A similarity to the relationship above discussed of needle nitrogen content to needle age, tree class and species, was observed in the relationship of photosynthetic and respiratory activities to these characters. For instance, with regard to ageing of needles in a dominant tree of *A. Mariesii*, the ratio in the total nitrogen content of 1-, 2-, 3- and 5-year-old needles was 100:87:84:78, and that in the light-saturated gross photosynthesis of the corresponding needles, 100:85:67:29, on a dry weight basis. Such a parallelism between these activities and the nitrogen content is well understood by linear relations between them shown in Fig. 9. This suggests that the physiological functions of needles are closely dependent on the amount of their protoplasm, which can be roughly assessed by total nitrogen content, as the major part of the latter may, according to Takeda and Maruta's results in rice plants[43]), be occupied by the protein-nitrogen.

The total nitrogen content in branchlets of various diameter is shown in Fig. 10,

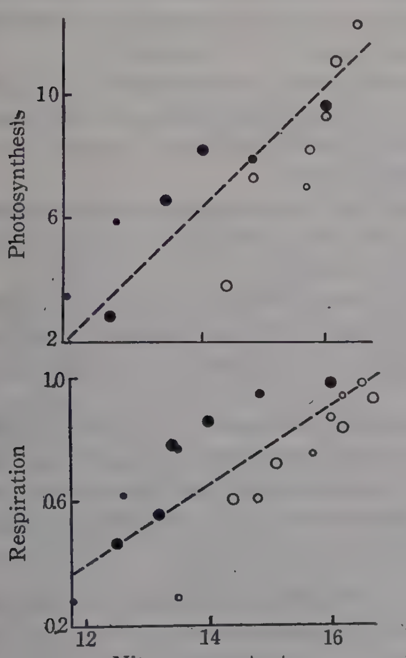


Fig. 9. Relation of nitrogen content of needle (mg./g. d. w.) to gross photosynthesis light-saturated and respiration, at 20° (mg. CO_2/g. d. w./hr.). Signs mean the same as in Fig. 6.

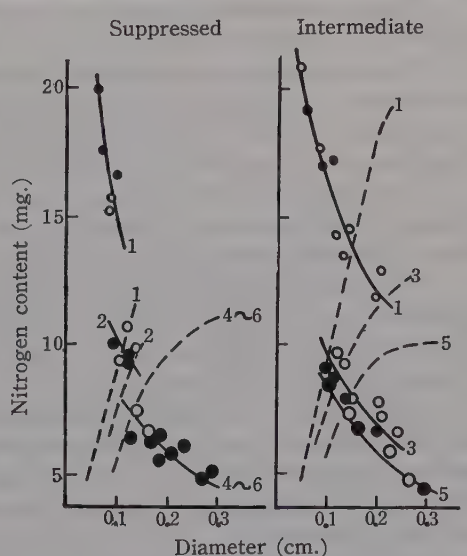


Fig. 10. Relation of nitrogen content of branchlet to its diameter (in dry state), per 1 g. dry weight of branchlet (solid lines) and per 50 sq. cm. surface area of branchlet (broken lines), in a 20-year-old *Abies* stand. Figures denote branchlet age. Open circles, *A. Veitchii*; solid ones, *A. Mariesii*.

being summarized in respect to branchlet age. The content per unit branchlet dry weight decreased with increase of branchlet diameter and age, and with size diminution of the trees. The nitrogen content per unit branchlet surface, however, increased with branchlet diameter increase. For instance, in the one-year-old branchlets with 0.05, 0.1 and 0.2 cm. diameter (dry) of the intermediate trees, the relative values of nitrogen content were on a weight basis 100:82:61, and on a surface basis 26:53:100. Such a relation of the branchlet nitrogen content to its diameter well accords with the already mentioned relation between respiratory activity and dimension of diameter in branchlet. Between the two *Abies* species there was no significant difference in the nitrogen content of branchlet, as in the case of its respiration. This parallelism between nitrogen content and respiratory activity in branchlet is quite reasonable as the respiratory functions are performed in the protoplasm where nitrogen is highly concentrated. It may also be expected, as observed in the distribution of respiratory activity in trunk of *Fraxinus nigra*[44]), that the nitrogen concentration in branchlet rapidly increases from the lowest value in the center to the highest in the cambium.

It has been brought into light that in the *Abies* trees, in accordance with the results of many other workers, the activity of physiological functions is closely related to the nitrogen content, and the more vigorous the trees are, the higher they are in their functions and nitrogen content.

Summary

Photosynthesis, respiration, needle thickness and nitrogen content were measured in the domidant, intermediate and suppressed trees in a 20-year-old *Abies* stand of the subalpine coniferous forest on Mt. Shimagare.

1. Needle thickness increased with needle ages and with size classes of the trees. In general, the thickness was larger in *A. Mariesii* than in *A. Veitchii*.

2. Photosynthesis and respiration decreased with ageing of the needles and with size diminution of the trees. The same trends were observed pertaining to the nitrogen content of needles. These functions and content were generally higher in *A. Veitchii* than in *A. Mariesii*, especially on a weight basis.

3. The needles of the dominant trees showed the characteristics of sun-leaves, and those of the suppressed, of shade-leaves, because these tree classes were situated in totally different light conditions within the *Abies* stand.

4. The leaf temperature optimal for gross photosynthesis was in the *Abies* species about 20°, which was fairly lower in comparison with the optimum temperature determined in broad-leaved evergreen trees in southern Japan, as accorded with the colder habitat of Mt. Shimagare.

5. Respiration and nitrogen content in branchlet decreased on a dry weight basis, but increased on a surface area basis, with its increasing diameter. In the respiration of entire trunk and root system, the intermediate (and dominant) was higher than the suppressed. Between the two *Abies* species there was little difference in all these measures.

The author's grateful thanks are due to Prof. M. Monsi and Prof. K. Hogetsu for their kind guidance under which this work was carried out.

References

1) Johansson, N., Sv. bot. Tidskr. **20**: 107 (1926). 2) Tschesnokov, V., und Bazyrina, K., Planta **11**: 473 (1930). 3) Boysen Jensen, P., Die Stoffproduktion der Pflanzen, Jena (1932). 4) Pisek,

A., und Tranquillini, W., Flora **141**: 237 (1954). 5) Lundegårdh, H., Klima und Boden, 4. Aufl., Jena (1954). 6) Tranquillini, W., Planta **46**: 154 (1955). 7) Böhning, R. H., and Burnside, C. A., Am. J. Bot. **43**: 557 (1956). 8) Kusumoto, T., Bot. Mag. Tokyo **70**: 299 (1957). 9) Kramer, P. J., The Physiology of Forest Trees, N. Y., 157, (1957). 10) Bormann, F. H., ibid., 197, (1957). 11) Nomoto, N., and Iwaki, H., Biol. Sci. (Jap.) **2**: 34 (1957). 12) Ichimura, S., and Aruga, Y., Bot. Mag. Tokyo **71**: 261 (1958). 13) Steemann Nielsen, V., Physiol. Plantarum **12**: 353 (1959). 14) Tazaki, T., Bot. Mag.Tokyo **72**: 68 (1959). 15) Kuroiwa, S., ibid. **72**: 413 (1959). 16) Stålfelt, M. G., Medd. Stat. Skogsf. **21**: 181 (1924). 17) Thomas, M. D., Ann. Rev. Plant Physiol. **6**: 135 (1955). 18) Freeland, R. O., Plant Physiol. **27**: 685 (1952). 19) Kusumoto, T., and Shinozaki, N., Bull. Educ. Res. Inst., Kagoshima Univ. **6**: 131 (1954). 20) Nixon, R. W., and Wedding, R. T., Proc. Am. Soc. Hort. Sci. **67**: 265 (1956). 21) Saeki, T., Bot. Mag. Tokyo **72**: 409 (1959). 22) Möller, C. M., Müller, D., and Nielsen, T., Forstl. Forsøgsv. Danmark **21**: 273 (1954). 23) Saeki, T. and Nomoto, N., Bot. Mag. Tokyo **71**: 235 (1958). 24) Pisek, A., und Winkler, E., Planta **53**: 532 (1959). 25) Oshima, Y., Kimura, M., Iwaki, H., and Kuroiwa, S., Bot. Mag. Tokyo **71**: 289 (1958). 26) Kusumoto, T., Jap. J. Ecol. **7**: 126 (1957). 27) Tranquillini, W., Ber. Deut. Bot. Ges. **67**: 191 (1954). 28) Walter, O. A., Flora **121**: 301 (1927). 29) Stålfelt, M. G., Planta **27**: 30 (1938). 30) Kusumoto, T., and Sakimoto, M., Bull. Educ. Res. Inst., Kagoshima Univ. **6**: 139 (1954). 31) Yamada, N., Murata, Y., Osada, A., and Iyama, J., Proc. Crop Sci. Jap. **23**: 214 (1955). 32) Murata, Y., and Osada, A., ibid. **27**: 12 (1958). 33) Müller, D., Planta **16**: 1 (1932). 34) Reinicke, A. J., Proc. Am. Soc. Hort. Sci. **32**: 77 (1934). 35) Müller, D., und Larsen, P., Planta **23**: 501 (1935). 36) Childers, N. F., Proc. Am. Soc. Hort. Sci. **35**: 253 (1937). 37) Batjer, L. P., and Degman, E. S., J. Agr. Res. **60**: 101 (1940). 38) Murata, Y., Osada, A. and Iyama, J., Proc. Crop Sci. Soc. Jap. **26**: 159 (1957). 39) Takeda, T., and Kumura, A., ibid. **26**: 165 (1957). 40) Tezuka, Y., Bot. Mag. Tokyo **71**: 181 (1958). 41) Loustalot, A. J., Gilbert, S. G., and Drosdoff, A. M., Plant Physiol. **25**: 394 (1950). 42) Murata, Y., Osada, A., Iyama, J., and Yamada, N., Proc. Crop Sci. Soc. Jap. **25**: 133 (1957). 43) Takeda, T., and Maruta, H., ibid. **25**: 120 (1956). 44) Godwin, R., and Goddard, D., Am. J. Bot. **27**: 234 (1940).

摘　　要

縞枯山の植生についての生態学ならびに生理学的研究
IV. *Abies* 幼樹の物質生産機能について

黒　岩　澄　雄

すでに報告された縞枯山のシラビソ・オオシラビソ第5森林の約20年生林分の優勢木，平均木，劣勢木の各器官について，同化能，呼吸能および全窒素含量を主に1957–8年にわたって夏測定した．

各階級木において針葉は老令化とともに厚くなるが同化・呼吸率は面積単位においてすら減少した．同一年令葉において針葉は階級木の劣勢化とともに薄くなるが，同化・呼吸率は重量単位でさえ減少した．針葉の厚さや同化・呼吸能についての各階級間での差異はいわゆる陰・陽葉間の差異に相当するが，これは各階級間での 群落内光環境 の差異にもとづいている．一般にオオシラビソより薄い葉をもつシラビソは重量単位ではオオシラビソより大きな同化・呼吸率を示したが，面積単位ではさほど大きな差はなかった．針葉の全窒素含量は，面積単位で葉令間差異が不明瞭だったほかは，同化・呼吸能と葉年令，樹木階級，樹種との関係に似て変化していた．またこれら針葉の全窒素含量と同化能や呼吸能との間にはほぼ直線的な関係があった．飽和光3万ルクスにおける平均木の各年令葉の光合成最適葉温は約20°で，楠元氏によって報告された鹿児島地方の常緑広葉樹の値よりかなり低く，縞枯山の寒い気候と一致していた．

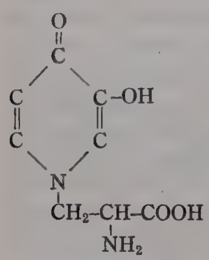
重量単位の小枝の呼吸率とその全窒素含量とは小枝の直径が大きいほど低いが，単位表面積あたりでは逆であった．また，小枝の窒素含量は小枝の直径が同じでも古い小枝ほど，また劣勢木のものほど含量は少なかった．幹全体の呼吸率は重量・面積両単位において，小枝と同様な直径関係が各階級木についてみられたが，劣勢木は常に最小値を示し，優勢木と平均木との間には差がなかった．根全体の重量単位呼吸率についても幹と同様な階級間差異がみられた．小枝の呼吸能と全窒素含量，幹や根の呼吸能については両樹種間ではっきりした差は認められなかった．（東京大学理学部植物学教室）

On the Physiological Properties of Mimosine

by Shôzô SUDA

Received October 5, 1959

Mimosa pudica L. is conspicuous because of the so-called sleeping movement which occurs under the influence of various stimuli. This quick and interesting movement is believed to be due to the turgor change of the pulvini of the plant. From the sap of the sprouts and roots of *M. pudica*, a substance has been extracted and designated as *mimosine* by Renz[1]). It was later demonstrated by Adams *et al.*[2,3,4,5]) and Brickel[6,7]) that mimosine is the same substance as leucenol extracted from ground seeds of *Leucaena glauca* Bentham, and is an amino acid, 3-hydroxy-4-keto-pyridylalanine. However, its physiological significance in *M. pudica* is hardly known. From the structure of mimosine found by Adams *et al.*[4]), the author expects that mimosine may have antimetabolic properties, since it contains a metabolite moiety (alanine), on the one hand, and a non-metabolite moiety (pyridyl) on the other.



Mimosine

Studies of mimosine have shown that it exerts an antagonistic action against some amino acids and indoleacetic acid in the growth of *Escherichia coli*. The substance is split in a few days by an enzyme, probably an induced one, of *E. coli*. Moreover, mimosine also proved to be split by an enzyme preparation which was obtained from pulvini of *M. pudica*. These reactions may play some role in the sleeping movement of the plant. In the present paper the antimetabolic properties of mimosine are dealt with.

Materials and Methods

Mimosine was prepared from the sap of *M. pudica* sprouts by means of a modification of Renz's method[1]). Drops dripping from the cut ends of the sprouts were collected. A considerable amount of precipitate soon appeared in the sap, but some contamination with small tissue debris and fine sand could not be avoided. A small amount of water was therefore added to the sap and the precipitate was dissolved by heating and filtered. Absolute alcohol was added until no more precipitate appeared. The precipitate was collected and washed with acetone and subsequently with 85% hot alcohol. The washed material was dissolved in hot water and the solution was concentrated under diminished pressure giving a crystalline solid. The raw crystals were separated by decantation before the solution became viscous. By several recrystallizations from water, a pure product (m.p. 228°) was obtained. The ninhydrin test and color reaction with ferric chloride or with Folin reagents agreed with those of both the mimosine reported by Renz and the leucenol of Adams *et al.* In the mixed melting point test with the isolated crystal and leucenol recrystallized from cold water, no depression of the melting point was observed. Therefore, the crystal was identified as mimosine and used in the present experiment.

The antimetabolic action of mimosine was tested by examining the growth inhibition of *E. coli* and its reversal by some metabolites. The B strain of *E. coli*, which

* Biological Institute, Faculty of Science, Kobe University, Kobe, Japan.

was maintained by daily serial transfer in a glucose-salt medium, was used for the test. The composition of the medium was as follows: $(NH_4)_2HPO_4$ 1 g., KH_2PO_4 0.5 g., NaCl 2.5 g., $MgSO_4 \cdot 7H_2O$ 0.1 g., glucose 2.5 g., distilled water 1000 ml. The reaction of the medium was adjusted with NaOH to pH 7.2. The growth experiments were carried out in the same medium.

As possible antagonistic metabolites against mimosine, phenylalanine, serine, proline, histidine, tyrosine, tryptophane and indoleacetic acid were examined, because of their similar structure and the suggested participation of auxin in the movement of *M. pudica*.

The growth of *E. coli* at 35° was measured turbidimetrically after 24 hours' incubation, the determination being made with a photoelectric nepherometer of Atago Optical Works. The growth inhibition is represented by the following expression:

$$\frac{\text{reading of control}-\text{reading of test solution}}{\text{reading of control}-\text{reading of uninoculated medium}}\times 100$$

The destruction of mimosine was tested by paper-chromatography.

Results

The expected action of mimosine in inhibiting the growth of *E. coli* is shown in Table 1. The antagonistic action against this inhibition is conspicuous in the case of

Table 1. Removal of antibacterial action* of mimosine by analogous amino acids and IAA.

Adjuvants		Mimosine ($\times 10^{-2}$ M): Per cent growth inhibition** none	1/64	1/32	1/16	1/8	1/4	1/2
None		0	−5	44	67	91	100	
DL-tryptophane	10^{-4} M	0	−2	23	43	72	93	
	10^{-3} M	0	0	2	11	24	47	85
L-proline	10^{-4} M	0	−2	33	53	81	100	
	10^{-3} M	0	−5	19	34	65	81	
L-histidine	10^{-4} M	0	−7	28	60	86	100	
	10^{-3} M	0	0	12	42	70	86	
L-tyrosine	10^{-4} M	0	−2	9	59	86	100	
	10^{-3} M	0	0	4	22	51	84	100
DL-phenylalanine	10^{-3} M	0	−2	31	60	87	100	
DL-alanine	10^{-3} M	0	0	12	35	77	100	
DL-serine	10^{-3} M	0	−2	11	33	81	100	
L-asparagine	10^{-3} M	0	−4	12	51	82	100	
IAA	10^{-6} M	0	−2	4	22	78	100	
	10^{-5} M	0	0	8	27	72	100	

* *Escherichia coli* was used as the test organism and incubated for 20 hours at 35°.
** See the text.

tryptophane. Tyrosine and proline also considerably neutralize the toxicity of mimosine. Histidine shows a little weaker action than the preceding two in the respect. Alanine, serine and phenylalanine antagonize to some extent, but asparagine to a less extent.

Increasing concentrations of added tryptophane, the strongest antagoniser of mimosine, were found to improve the growth inhibited by mimosine. At a tryptophane concentration of 10^{-3} M, removal of inhibition was remarkable, but at a concentration of 10^{-4} M, its effect was less, and at 10^{-5} M there was no effect whatever. In the second-ranking group of antagonisers, tyrosine, proline and histidine, the titers of antagonizing action were always about half that of tryptophane, but these effects mostly disappear at a concentration of 10^{-4} M.

When IAA was added to the medium at concentrations of 10^{-6} M to 10^{-5} M, that is, concentrations actually found in plant tissues (except in roots), the growth inhibition by mimosine at concentrations less than M/1600 was prevented in a non-competitively antagonistic way, according to the formula proposed by Shive[8]).

On the other hand, growth inhibition by lower concentrations of mimosine disappeared after a few days' incubation without addition of any metabolite. It was inferred that the detoxification of mimosine was caused by the destruction of the substance by an enzyme (probably adaptive) formed by *E. coli*. The cultures in which the organisms grew in spite of the presence of mimosine were therefore filtrated through a Seitz-filter, and the bacterium-free filtrate was evaporated to dryness under diminished pressure. The residual matter was then extracted with 95% ethanol. The ethanol solution was concentrated and partitioned by paper-chromatography. The solvent systems used were (A) butanol-acetic acid-water (4:1:2 by vol.) and (B) phenol-water (100:23 by vol.). Toyo Roshi's No. 52 paper was used. All runs were carried out at room temperature. The developed paper was sprayed with ninhydrin solution to develop a purplish orange reacting with mimosine. The R_f value was about 0.12 in the case of solvent system A, and about 0.51 in B. The spots corresponding to alanine and serine were also detected, together with a distinct spot of a yet undetermined substance in both solvent systems (Fig. 1). These points were not detected in the cultures without mimosine.

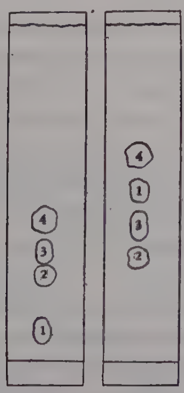
These results suggest that mimosine is split by *E. coli* into alanine or serine and probably pyridyl residue. The hydrolysis of mimosine with hydrochloric acid or barium hydroxide was therefore tried. No spot corresponding to serine was detected on the paper-chromatogram obtained with either hydrolysate, although a spot corresponding to alanine was evident.

It will be natural to suppose that in the mimosa plant which produces mimosine in significant amounts an enzyme (or enzymes) synthesizing or decomposing this substance must be present.

Therefore, 10 g. of the excised pulvinus material was ground in a cooled blender with 30 ml. of phosphate buffer of pH 6.0, and the homogenate was centrifuged at 5000 *g* for 10 minutes. The supernatant solution was saturated with ammonium sulfate, giving an amorphous precipitate. This was collected and suspended in 10 ml. of cold distilled water, and dialysed overnight at 4°. The slightly turbid solution obtained was used as the enzyme solution. As the precipitate made by adding acetone to the solution proved to be less active than the original solution, the procedure was not adopted.

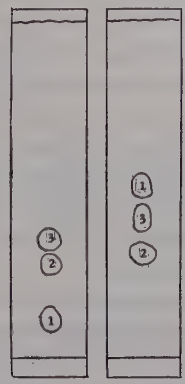
Four different reaction mixtures of varied pH values were prepared. 0.5 ml. of the enzyme solution, 0.5 ml. of 1/400 M mimosine solution and 0.5 ml. of buffer solution of pH 5.0, 6.0, 7.0, or 8.0, were mixed in test tubes. Phosphate buffer could not be used in this experiment since, in the subsequent paper-chromatogram developed with solvent system A, it caused a faint yellow spot to appear just below alanine on spraying with ninhydrin. Lillie's citrate and Michaelis' barbital buffer were therefore

used. The control series without mimosine or the enzyme were also run. After standing for an hour at room temperature (about 28°), each reaction mixture was spotted on the paper, and developed with solvent system A or B. In the lot of pH 8.0, the spot corresponding to serine was clearly detected, although two other ninhydrin positive spots were also detected (Fig. 2), one corresponding to mimosine and



A B

Fig. 1. Paperchromatograms A and B showing typical separation of alcoholic extract of mimosine-containining culture filtrate of *E. coli*. Strips A and B were developed for 18 hrs., in solvent systems A and B, respectively. Spots correspond to minosine (1), serine (2), undetermined substance (3) and alanine (4).



A B

Fig. 2. Paperchromatograms of ninhydrin positive substances present in the reaction mixture of mimosine and mimosa enzyme at pH 8.0. A, B, 1, 2 and 3 indicate the same as in Fig. 1.

the other to yet undetermined substance shown in the case of *E. coli*. In neutral or acidic reaction, these spots were very faint except for that of mimosine, and in control lots, the spots corresponding to serine and the undetermined substance were never detected.

Discussion

Sibaoka[9,10] has recently reported the results of investigations on the excitatory conduction in *M. pudica*, and Toriyama[11,12,13] has studied the cytology of the pulvinus, or petiole, of *M. pudica*. The role of mimosine in the variation movement of the plant, however, remains obscure.

In the present study, mimosine has proved to exert an antimetabolic action on *E. coli* which is conspicuously antagonized by such heterocyclic amino acids as tryptophane, proline and histidine. It is interesting that the antagonistic activities of these heterocyclic compounds are stronger than those of phenylalanine, alanine and serine. From these results, it is inferred that the active site of mimosine involved in its inhibitory action is the pyridyl moiety. The hydroxyl-group may also play a certain role, since tyrosine strongly antagonizes the toxic action of mimosine in spite of its non-heterocyclic nature whereas phenylalanine does so only weakly.

The competitive nature of the antagonism between mimosine and tryptophane, although likely, has not been definitely established.

Lansfield and Shive[14]) reported that 2-pyridinealanine (i.e., 2-pyridylalanine) inhibits the growth of *E. coli* and phenylalanine prevents such inhibition, and that tryptophane reverses the inhibition, thus indicating that it stimulates the biosynthesis of phenyl-

alanine in this organism. Tyrosine, on the other hand, is shown to exert only a slight effect in reversing this toxicity. These results differ from ours in that phenylalanine shows only a slight antagonistic effect against the growth inhibition by mimosine, whereas both tryptophane and tyrosine are antagonistic to mimosine. The position of the pyridine-alanine linkage and of the hydroxyl-group in the pyridyl residue may explain the difference between these results.

It is interesting that both alanine and serine were chromatographically detected in the culture filtrate of *E. coil* when mimosine was added to the medium, whereas the latter alone was detected when mimosine had been treated with the enzyme solution prepared from the pulvini. In another experiment to be reported elsewhere in detail, mimosine and serine, but not alanine, were detected in the sap of the mashed pulvini which is considered to contain the product of the enzyme reaction. Therefore, the difference probably is not due to the absence of some co-factors in the purified preparation of mimosa enzyme, but to the difference in property of the bacterial enzyme.

It is also interesting to note that serine, which antagonizes, although slightly, the bacterial growth inhibition exerted by mimosine, is formed by the action of an enzyme in the mimosa pulvini. Guttenberg and Kröpelin[15]) have explained that the variation movement of *Phaseolus coccineus* is closely connected with auxin in the pulvini which causes the increase of water permeability of the pulvinus cells. In the present experiment, IAA proved to counteract the growth inhibiting action of mimosine toward *E. coli*. It seems, therefore, likely that the antagonism between IAA and mimosine may exist also in the mimosa pulvini.

These points may have some applicability in the consideration of the mechanism of the sleeping movement of the mimosa plant.

Summary

1. Mimosine inhibits the growth of *E. coli*, and the inhibition is antagonized by tryptophane, proline, histidine, tyrosine, phenylalanine, alanine, serine and IAA.

2. The growth inhibition by mimosine disappeared on incubating for a few days. This loss of the antibacterial action is probably due to the destruction of mimosine by an enzyme (probably adaptively) formed by *E. coli*. Alanine and serine were detected chromatographically in the cultures which contained mimosine.

3. An enzyme preparation is obtained from the pulvini of *M. pudica*, which splits mimosine. A spot corresponding to serine is paper-chromatographically detected from the reaction mixture. The optimal pH is about 8.

4. In view of the result obtained, the mechanism of sleeping movement in the mimosa plant was discussed.

The author wishes to express his appreciation to Prof. M. Nagao for his valuable advice and for reading the manuscript, to Prof. T. Jimbo for his criticism and to Prof. M. Shibata and Dr. T. Sibaoka for the supply of leucenol.

References

1) Renz, J., Zeitschr. physiol. Chem. **244**: 153 (1935). 2) Adams, R., Cristol, S. J., Anderson, A. A., and Albert, A. A., J. Am. Chem., Soc. **67**: 89 (1945). 3) ——, and Jones, V. V., ibid. **69**: 1803 (1947). 4) ——, ——, and Johnson, J. L., ibid. **69**: 1810 (1947). 5) ——, and Johnson, J. L., ibid. **71**: 705 (1949). 6) Brickel, A. F., ibid. **69**; 1801 (1947). 7) ——, ibid. **69**; 1805 (1947).

8) Shive, W., Proc. Ann. N. Y. Acad. Sci. **52**: 1212 (1950). 9) Sibaoka, T., Sci. Rep. Tohoku Univ. 4th Ser. (Biol.) **19**: 133 (1951). 10) ——, ibid. **20**: 139 (1954). 11) Toriyama, H., Cytologia **18**: 285 (1953). 12) ——, ibid. **19**: 29 (1954). 13) ——, Bot. Mag. Tokyo **71**: 309 (1958). 14) Lansfield, E. M. Jr., and Shive, W., Arch. Biochem. Biophys. **38**: 347 (1952). 15) Guttenberg, H. v., and Kröpelin, L., Planta **35**: 257 (1947).

摘　要

ミモシンの生理学的特性について

須　田　省　三

Adams 等によって提示されたオジギソウ成分ミモシンの構造式からミモシンは代謝拮抗物質としての性質をもっているものと予想されたので，ミモシンの抗菌性を調べたところ，1/3000 M 以上の濃度では大腸菌の生長に対して抑制的に作用することが観察された．しかしてこの抗菌性はトリプトファン，プロリン，ヒスチジン，チロシン，フェニールアラニン，アラニン，セリンあるいはインドール酢酸の存在によって喪失または軽減されることが明かにされた．ところが，これらのミモシン拮抗物質を添加しなくてもミモシンによって抑制されていた大腸菌は低濃度区では培養数日後に生長を回復してきた．これは大腸菌の形成した適応酵素による解毒作用の結果，ミモシンが分解されたものと考えられ，このような培地中にはアラニンおよびセリンと思われる物質の存在がペーパークロマトグラフィーによって明らかに検出された．オジギソウ主葉枕から得た粗酵素標本によるミモシンの分解が同様にペーパークロマトグラフィーによって検討されたが，この場合はセリンに相当する物質とニンヒドリン陽性未決定物質（大腸菌による場合にも検出された物質）のみが検出された．この酵素反応の最適 pH は約 8.0 にあるようである．以上の実験結果からオジギソウの睡眠運動においてミモシンが重要な役割を演ずるであろうことが論述された．（神戸大学理学部生物学教室）．

Studies on the Dehydration Resistance of Higher Plants I
Determination of the Measures Related to the Dehydration Resistance of Mulberry Plants*

by Tadayoshi TAZAKI**

Received October 13, 1959

The growth of pine yearlings in dune regions was investigated in a previous paper[1]) with special reference to their drought resistance. The term, drought resistance, was then defined as the ability of plant to survive drought condition by enduring the water loss when water absorption by root practically ceased due to the exhaustion of available water in their rhizosphere. Different from drought resistance the author wishes to define the term, dehydration resistance, as the ability of plant parts or a whole plant to bear water loss after unnatural treatments such as detaching plant parts, digging out whole plants and so forth. Then, dehydration resistance will serve as the first step of the analysis of drought resistance, though in the latter the water supply for leaves from other plant parts yet continues even if the water absorption by root already ceased. The study of dehydration resistance also has practical importance in cultivated plants in which detached plant parts are utilized for agricultural purposes, e.g., mulberry plant for sericulture, tobacco plant, forage plant and so forth.

The author has made an attempt to express dehydration resistance quantitatively as the time required from detaching to kill the plant part by water loss under a given humidity condition. To date investigations have been meagre for this line of study in water economy of higher plants. Huber[2]) defined drought resistance as the ratio of transpiration to water absorption expressing both amounts by various measures concerned, while Walter[3]) approached it from the osmotic relations of plant parts. But their investigations were not conducted from the viewpoint of water economy, i.e., the arithmetic consideration of transpiration, water absorption and water amount of plant parts. Fukuda[4]) applied some experimental formulae to the time trend of transpiration after detaching leaves, but did not go further. Recently, Satoo[5]) expressed by a formula the drought resistance of three species of conifer yearlings by a similar procedure of the author.

Before entering the analysis with mathematical formulae, some results of experiments will be mentioned for determining measures related to the dehydration resistance of mulberry leaves, as no result of investigation has been reported concerning the water economy of this plant. As mulberry leaves are successively unfolding throughout the growing season the variation of those measures with leaf age must be taken into account in addition to daily or seasonal variation as was the case in the study of carbon dioxide assimilation in this plant[6]). Only the dehydration resistance was pursued of mulberry plant after detaching leaves for the present step, as this plant has a large mass of stem, stump and root, the dehydration resistance of intact leaves,

---

* "Dull" leaves in mulberry plants, a part of this paper, were already reported at the 17th General Meeting of the Botanical Society of Japan in 1952.

** Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan.

consequently, is a more complex problem. Also the water loss after detaching mulberry leaves has much bearing on the practical aspect of silkworm rearing, especially on the maintenance of adequate water content as the feed stuff for silkworm.

The material for this study was Kairyo-nezumigaeshi, one of the commonest forms of mulberries (*Morus alba*) cultivated at the mulberry field of Tokyo University of Agriculture and Technology situated at Koganei, Tokyo. The plants were trained in the "root cut" type, planted and manured in the conventional manner as was mentioned in a previous paper[6]). Leaves of "spring cut" mulberries as well as of "summer cut" ones were used for the experiments, which were commenced in the summer of 1951 and were continued till 1958. Most of the experiments were repeated more than twice in different year in order to ascertain the yearly fluctuation. Data obtained during the summer time were chiefly mentioned in the following, as the dehydration resistance in this season is much of interest from ecological as well as sericultural viewpoint.

1. The amount of transpiration after detaching leaves:

Detached leaves were hung to the torsion balance immediately after detaching, mostly in two minutes, the amount of transpiration was then determined by weight loss during a definite time interval which was between 2 and 60 minutes in accordance with transpiration rate. As the weight of a mulberry leaf is too heavy to be measured by the torsion balance, a leaf part of the size, 2 cm. $\times$ 10 cm., without midrib and large veins, was cut from the right or left half of a lamina, the cut end was then immediately sealed with vaseline before it was hung to the balance. The amount of transpiration was converted to relative transpiration (T_r) by measuring the evaporation of leaf-shaped evaporimeter, a moistened filter paper lined with aluminium foil, as the stomata of this plant are found only upon the lower side of leaves. As it was made clear by a preliminary experiment that there existed no significant difference between a whole leaf and a leaf part in the value of T_r immediately after detaching as well as in the variation afterwards, it may not be hazardous to apply the results obtained in leaf parts to whole leaves.

We shall begin by making clear how initial T_r, the value of T_r immediately after detaching, varies during the daytime. For example, on a fine day in summer the leaves were frequently, mostly every five minutes, detached and the amount of transpiration was measured together with the water content of leaves and the atmospheric saturation deficit in mulberry field. Young mature leaves, the 10th from shoot apex, were selected for the measurement in order to make uniform the condition of leaf age. The result was that T_r rapidly increased with sunrise and attained to a maximum value as early as 7hr., then the value decreased slowly until 17hr., falling abruptly with sunset. The variation of leaf water-content took opposite trend, diminishing with time till 16hr. with a minimum value of 260% on an oven dry basis, and then the value began to increase towards evening (Fig. 1). The daily march of stomatal aperture verified by benzol infiltration also took similar course as that of T_r. On the basis of these results the experiments of the variatian of T_r after detaching leaves were done between 9 and 14hr. when the stomata were open widely.

The maximum value of T_r in mulberry leaves was equal to, or a little larger than 100%, i.e., mulberry leaves transpire from both side as much as water vapour as one-sided moistened filter paper. This value is extraordinarily high compared with the values, 60% or thereabouts at maximum, for various land plants of Japan in Monsi's paper[7]). Only the values of some water plants and wheat plant in his data

are comparable. The difference by leaf order or leaf age of the initial T_r was insignificant during the daytime, i.e., every leaf on a shoot showed high values simultaneously. Next, the value of relative cuticular transpiration (T_{rc}) was measured for leaf upper side without stomata by sealing the lower side with vaseline. The values of T_{rc} were small in older leaves and large in younger ones, ranging between 5 and 10%. As the T_r at stomatal closure, i.e., 7–14%, was about 1.4 times of the above value, the T_{rc} of lower side may be conjectured to be equal to that of upper side, because both side of evaporating surface with equal evaporating power ordinarily

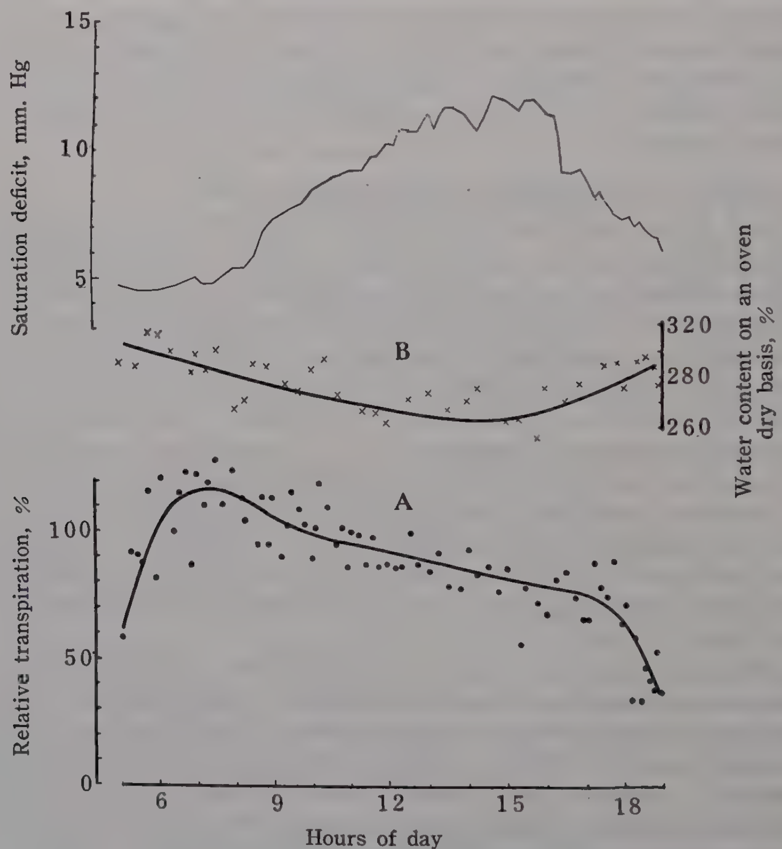


Fig. 1. Daily variation of A) relative transpiration and B) leaf water-content in the 10th leaf of "summer cut" mulberries, Kairyo-nezumigaeshi, measured on Aug. 11, 1952, a fine day

evaporates 1.4 times of the amount for one-sided evaporating surface. In addition the amount of cuticular transpiration *per se* for lower side was ascertained by sealing the upper side at stomatal closure.

The value of T_r in mulberry leaves fell to less than 20% after about 30 min. from detaching with closure of stomata. However, in some mulberry leaves the amount of T_r remained comparatively large with open stomata at long interval from detaching. It was revealed that this phenomenon had much bearing on leaf age. For example, the variation of T_r after detaching leaves was followed on July 21 in a "spring cut" mulberry plant (Fig. 2). Numbering from shoot apex the leaves till

the 35th showed small value of T_r in a lapse of time, whereas in older leaves lower than the 36th the large amount of T_r, 40% or thereabouts, persisted after long interval from detaching. This large value was evidently due to the imperfect closure of stomata as was verified from the direct observation of stomatal aperture and the small value of T_{ro}, which was below 10% without exception. These mulberry leaves with inert closure of stomata by hydroactive movement were named " dull " leaves by the author. As " spring cut " mulberries had about 60 leaves on one shoot at

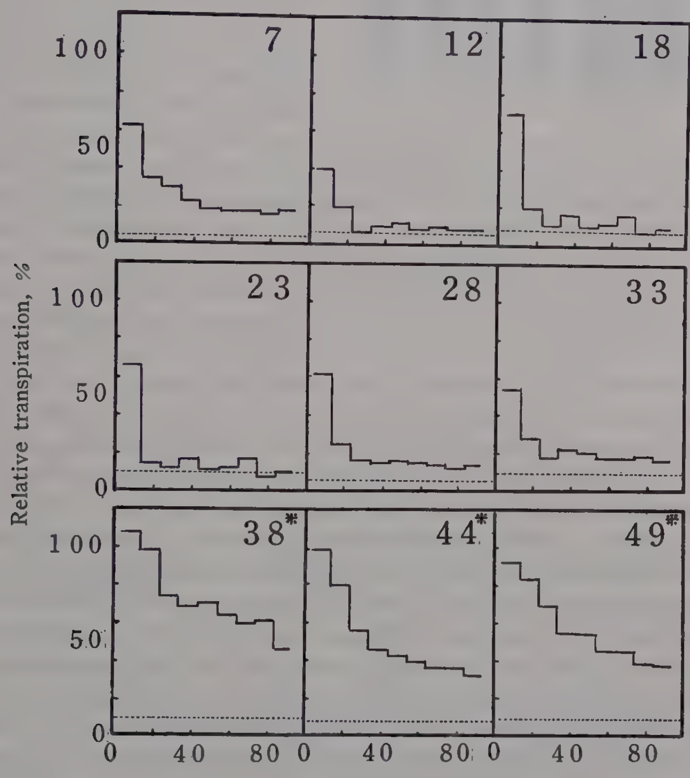


Fig. 2. Variation of relative transpiration after detaching leaves measured almost simultaneously on July 21, 1952 for various leaf order (numbers in the figure) in a shoot of " spring cut " mulberry. The numbers marked with sign, *, are " dull " leaves. Broken lines show the culicular transpiration of upper side with no stomata.

this date, almost half leaves had been converted to " dull " leaf till that time. The discrimination of both kinds of leaves can easily be done by hanging the detached leaves in the laboratory. Then " dull " leaves are perfectly killed after definite interval by their large water loss, while normal leaves are kept in fresh condition on account of the small transpiration rate due to stomatal closure. The condition of July 21, above mentioned, was shown by a photograph (Fig. 3). The boundary between both types of leaves became higher and higher as the progress of the season. Thus on September 10 in the same year leaves older than the 15th were already turned to

"dull" in a "spring cut" mulberry shoot with 85 leaves. The first appearance of "dull" leaf was observed at the middle of June in "spring cut" mulberries, so it has become clear that the leaves unfolded in early spring turned to "dull" after two months from unfolding, as mulberry leaves begin to unfold at the middle of April in Tokyo. But the leaves unfolded in early summer and early autumn, it was made clear, turned to "dull" leaf within a month from unfolding. The shortening with the progress of unfolding date of the duration in normal condition concerning hydroactive reaction was much of interest in the parallelism between the variation with unfolding season of the duration in maximum rate of photosynthetic activity for mulberry plant[6].

Fig. 3. Photographical illustration of normal and "dull" leaves in the leaf parts whose relative transpiration was measured on July 21, 1952 (Fig. 2). Leaf parts were hung indoors after the measurement of T_r, and was photographed after 5hrs. from detaching. The 7th–33rd leaves were normal and the 38th–49th "dull".

The same sort of experiment was carried out for "summer cut" mulberries also at the end of July, 1952. Before the experiment it was expected that nearly all leaves were yet normal, as they had unfolded after "summer felling" at the beginning of June. Contrary to the expectation, however, out of 22 leaves on July 25 for one shoot, only 6 leaves near the apex were normal, the remaining 16 leaves were already "dull", and at the beginning of autumn nearly all leaves had been turned to "dull" leaves except for the youngest unfolding leaves. The difference between "spring cut" and "summer cut" shoots was so amazing that the author attempted to investigate its reason. The result of some approaches to this problem will be mentioned in the following section.

In autumnal night the aperture of stomata was examined in mulberry plants in the field by benzol infiltration, and it was observed that the stomata remained open in "dull" leaves, while they were perfectly closed in normal ones, showing that the photoactive reaction for stomatal movement also became inert in "dull" leaves. The situation was quite the same in some experiments done during summer. Besides, in some deciduous tress, e.g. in *Zelkowa serrata* and *Ginkgo biloba*, the stomata were also open during autumnal night. Nocturnal opening of stomata was reported by several investigators, e.g., von Faber[8]) in tropical plants and Stålfelt[9]) in arctic plants. From above observations it seems that the opening of the stomata during the night time, especially in autumn, may be an universal phenomenon at least concerning some deciduous trees in temperate zone. Quantitative considerations for the relation between T_r and the time after detaching leaves will be mentioned in the following.

2. The water content of mulberry leaves:

The commom pattern of the variation of water content in successive leaves with different leaf age was that the water content was maximum at the youngest leaf at shoot apex, and abruptly decreased at a few leaves just under the youngest one, keeping nearly a constant value from younger to mature leaves at the middle part of the shoot with somewhat increase at the lowermost leaves (Fig. 4). For "spring cut" shoots in midsummer, the water content of the youngest leaf was usually over 400% on an oven dry basis, in some cases even more than 500%, the water content then rapidly

decreased till the 10th leaf from the apex, the values being kept nearly constant, i.e., 230–270%, for wide range of mature leaves. Similar trend was also observed in "summer cut" shoots during summer at least on an oven dry basis. It must be remembered, however. that the water content on a leaf area basis (mg./cm².) has more direct bearing on the water loss of leaves than that on an oven dry basis. In order to calculate the water content on a leaf area basis the areal weight of leaves in mg. dry weight for cm². was measured in "spring cut" as well as in "summer cut" shoots simultaneously with the determination of water content on an oven dry basis. The areal weight of mature leaves in "spring cut" shoots during summer was 5 mg./cm². or thereabouts, and that in "summer cut" shoots a little smaller, i.e.,

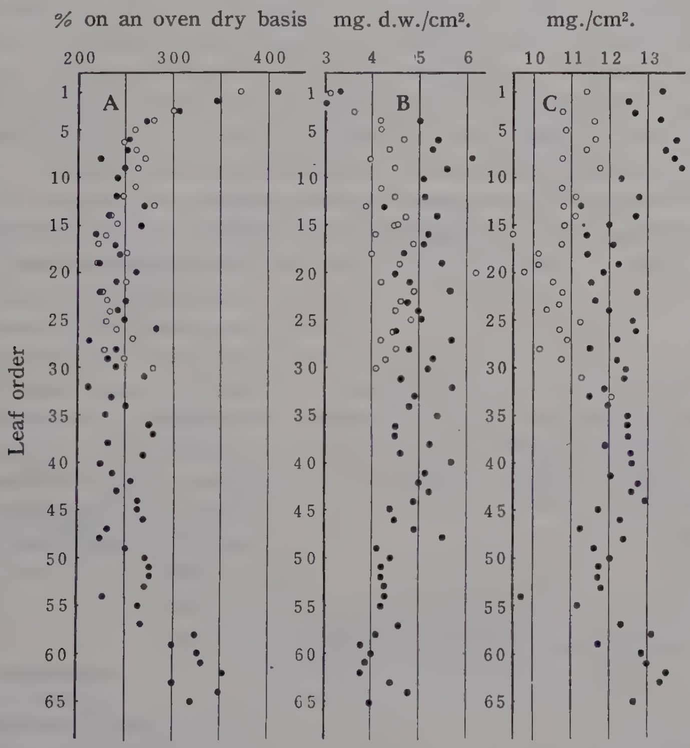


Fig. 4. Water content on an oven dry basis (A), leaf areal weight (B) and water content on a leaf area basis (C) for successive leaves of "spring cut" (●) and "summer cut" (○) shoot of mulberry plant measured on Aug. 12 and Aug. 2, 1958, respectively.

4.5 mg./cm². In both shoots the youngest leaves showed smaller values. Owing to a reversible relation in the leaf order trend between the water content on an oven dry basis and the areal weight, the water content on an areal basis showed comparatively constant values, thus about 12 and 11 mg./cm²., respectively, for "spring cut" and "summer cut" shoots. Small amount of water content on a leaf area basis may be one possible cause of "dull" leaf, as it is often followed also by the small amount of lethal deficit, arousing a possibility of withering before the complete closure of stomata by hydroactive reaction, and of the stomata remained open after death, though this explanation may not be so promising in the different behaviour for both training types of becoming "dull" leaf because of the small difference in

water content and therefore in lethal deficit, for the lethal water content was almost the same in both training type as will be mentioned in the following section.

3. The lethal water content and lethal deficit of mulberry leaves:

For the mathematical examination of dehydration resistance we must know the lethal deficit of leaves. Lethal deficit is, as was mentioned previously, the difference of water content and lethal water content. At first the author attempted to determine lethal water content both by the recovery of turgescent state after soaking the petiole of detached leaf into water, and by colour reaction with benzidine. As both attempts failed he was obliged to apply plasmolytic method, though not preferable. Thus, small leaf parts without large veins (ca. 5 mm.×5 mm.) were soaked into 1N solution of NaCl and after suitable interval, mostly 30 min., the occurrence of plasmolysis was observed by penetrating light at small veins with no chloroplast. In living cells plasmolysis occurred without fail, as the concentration of NaCl solution isotonic with the cell sap was about 0.3N. Then the vitality of leaf cells could be examined with the success or failure in plasmolysis. In addition the water content of leaves was measured at the same time, by which it became possible to determine lethal water content. An example for mature leaves is illustrated in Fig. 5. The values of lethal water content thus determined for younger, mature and older leaves in both training type during summer were, respectively, 190, 120 and 120% on an oven dry basis. So the lethal deficit was 60, 130 and 130% if we assigned initial water content as 250%. As is observed from the figure the determination was not so clearcut as in the case of pine yearlings[1]).

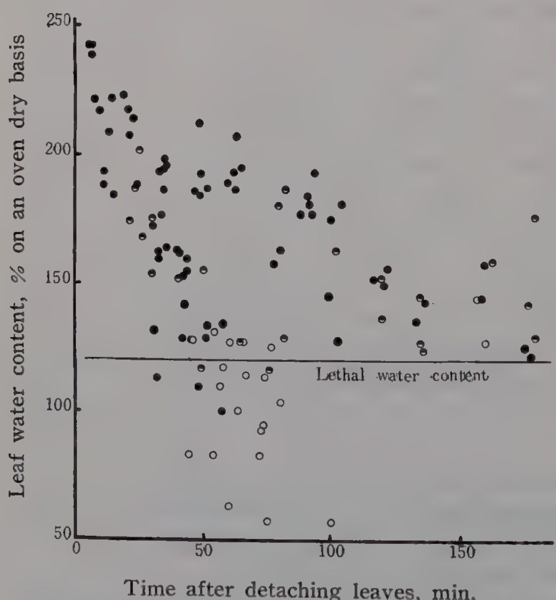


Fig. 5. Determination of lethal water content in mature leaves of "spring cut" mulberries by the plasmolytic method. The points, ● ◓ ○ are the water content of living, half killed and killed leaves respectively.

Summary

By dehydration resistance the author means the ability of plant parts or a whole plant to bear water loss after unnatural treatments such as detaching plant parts or digging out whole plants. Before entering analysis with mathematical formulae, some results of experiments were mentioned for determining measures related to the dehydration resistance of mulberry leaves.

1. Relative transpiration of mulberry plant was large throughout the daytime with open stomata. Maximum relative transpiration for the one-sided evaporimeter was over 100%, which was only comparable with some water plants. Cuticular transpiration for one side was below 10%, while relative transpiration at stomatal closure

was about 1.4 times of the former amount.

2. Concerning hydroactive closure of stomata at diminished water content of leaves after detaching, "dull" leaves were found with inert stomatal closure. This phenomenon has much to do with the age of leaves, but the situation was quite different in "summer cut" and "spring cut" mulberries.

3. The common pattern of the variation of water content on an oven dry basis by leaf order or leaf age during summer was that the water content was maximum at the youngest leaf at shoot apex, mostly over 400%, and diminished abruptly at a few leaves just under the youngest one, being kept nearly constant, about 250%, from younger to mature leaves at middle part of the shoot with slight increase at lowermost leaves. The areal weight and the water content on a leaf area basis in mulberry leaves during midsummer were 5 mg.d.w./cm². and 12 mg./cm². for "spring cut" mulberries and 4.5 and 11 in the same unit for "summer cut" mulberries, respectively.

4. Lethal water content determined in summer for younger, mature and older leaves were, respectively, 190, 120 and 120% on an oven dry basis. So the lethal deficit was 60, 130 and 130% if we assumed initial water content as 250%.

The author wishes to express his cordial thanks to Prof. Messrs. Monsi, the University of Tokyo, and Prof. K. Hôgetsu, Tokyo Metropolitan University, for their kind advice and criticism throughout this investigation. Thanks are also due to Messrs. T. Ushijima and T. Murakami to their help for preparing the text.

References

1) Tazaki, T., Jap. Journ. Bot. **17** (2): 240 (1960). 2) Huber, B., Jahrb. f. Wiss. Bot. **64**: 1 (1924). 3) Walter, H., Die Hydratur der Pflanzen und ihre physiologisch-ökologische Bedeutung. Jena (1931). 4) Fukuda, Y., Pflanzenforschung Heft **19**: 1 (1933). 5) Satoo, T., Bull. Tokyo Univ. Forests No. 51: 1 (1956). 6) Tazaki, T., Bot. Mag. Tokyo **72**: 68 (1959). 7) Monsi, M., Jap. Journ. Bot. 8: 97 (1944). 8) von Faber, Fr. C., Jahrb. f. Wiss. Bot. **56** (1915). 9) Stålfelt, M., Svensk. bot. Tidskr. **19** (1925).

摘　　　要

高等植物の乾燥抵抗に関する研究 I
クワの乾燥抵抗に関する諸量

田　崎　忠　良

高等植物の乾燥抵抗を乾燥死するまでの時間として表現し，いろいろ考察をくわえた．乾燥抵抗とは，植物が人工的な処理をうけたのち，水の消失に対する抵抗能力として定義した．クワを中心とする乾燥抵抗を論議するまえに，その乾燥抵抗算出に必要な諸量を決定する実験についてのべる．

1．クワの比較蒸散量 (T_r) は，気孔が日中はひらいているので，昼間はつねにおおきい．T_r の最高値は 100% 以上にもなり，水生植物に相当する．片面のクチクラ蒸散量は 10% 以下であり，気孔のとじたばあいの T_r はその約 1.4 倍である．

2．摘葉後水の消失にともなう気孔の水能動閉鎖運動については，その閉鎖が不活発ないわゆる「鈍葉」(dull leaf) がみられた．この現象は葉令に関係があり，「春ぎりクワ」と「夏ぎりクワ」では様相をことにする．

3．夏時葉位にともなう対乾物含水量変化の普通な型はつぎのとおりである．含水量は先端のいちばんわかい葉で最大であり，400% 以上にもなり，そのしたの数葉で急に減少し，枝の中部の葉ではほぼ 250% である．成熟した葉の面積重および面積あたりの含水量は，「春ぎりクワ」では 1 cm² あたり 5 mg および 12 mg であり，「夏ぎりクワ」では 4.5 mg および 11 mg であった．

4．夏時幼葉，成熟葉および老葉の致死含水量はそれぞれ 190，120 および 120% であり，最初の含水量を 250% とすれば，致死飽差（最初の含水量と致死含水量の差）はそれぞれ 60，130 および 130% になる．（東京農工大学繊維学部）

Aspergillus の醱酵と発芽に関する生理学研究

II. *Aspergillus awamori* の浸透価とプロティナーゼ，α-アミラーゼの生成との関係

高　見　　亘*

Wataru TAKAMI*: Physiological Studies of *Aspergillus* with Special Reference to the Fermentation and the Germination
II. Relation between Osmotic Value and Formation of Proteinase and α-Amylase in *Aspergillus awamori*.

1959 年 8 月 1 日受付

糸状菌のプロティナーゼについては多くの酵素学的研究があり，とくに *Aspergillus* 属のものが最も詳細に研究され，最適 pH によって *niger* 型の酸性側プロティナーゼと *oryzae* 型のアルカリ側プロティナーゼに分けられ[1]それぞれ結晶化された[2,3]．それらの酵素学的特性も詳しく研究されている[4,5,6]．また．糸状菌のアミラーゼの酵素学的研究も多いが[7,8]，前報[9]で試みたような細胞の浸透価に関係した研究は未だなされていない．ここではこのような見地から行なった実験の結果を報告する．

材料および方法

使用菌株は，プロティナーゼ研究のためには，酸性沪紙法によって分離した約 80 株のうちの一株 No. 82 の *Aspergillus awamori* を用いた．この株はしょ糖，ぶどう糖，果糖などの糖を含む合成培地で多くの有機酸を蓄積し，強力な耐酸性のプロティナーゼを生成するが，このような条件の下ではアミラーゼは全く生成しない．また，アミラーゼ研究のためには公式菌株 *Aspergillus awamori* HUT 2014 を用いた．

プロティナーゼ研究のための培養基はつぎのごとくである．

しょ糖	100 g
NH_4NO_3	2 g
KH_2PO_4	2 g
$MgSO_4 \cdot 7H_2O$	0.25 g
$MnSO_4 \cdot 4H_2O$	0.02 g
$FeCl_3 \cdot 6H_2O$	0.024 g
$ZnSO_4 \cdot 7H_2O$	0.005 g
ゼラチン	0.33 g
蒸留水 (pH 5.8)	1000 cc

また，アミラーゼ研究のためには Czapek 液

しょ糖	30 g
$NaNO_3$	2 g
K_2HPO_4	1 g
KCl	0.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$FeSO_4$	0.01 g
蒸留水 (pH 5.8)	1000 cc

および，しょ糖を等量のぶどう糖，デキストリンに代えたものを用いた．

上記の培養液を，表面培養では 100 cc フラスコに 30 cc ずつ分注し，1 白金耳の胞子を接種，30° の恒温器中で培養し，深部培養では 500 cc の振盪用フラスコに 80 cc ずつ分注し，3 白金耳の胞子を接種，30° で毎分約 110 回転で振盪し，毎回 2 個ずつのフラスコをとり出してその沪液をプロティナーゼ，α-アミラーゼの測定に供した．

作用力測定法はプロティナーゼでは Folin 試薬を用いる Anson 法の荻原による改変法[10]によった．すなわち M/5 乳酸溶液に 2 %濃度に溶解したカゼイン溶液 1 cc に，pH 3.2 に調整した培養沪液 1 cc を添加，55° で 1 時間反応させ，0.4 M トリクロル酢酸 2 cc を加え反応を停止させ，活性度は生成した 0.4 M トリクロル酢酸可溶の Folin 呈色物質の 660 mμ における吸光係数を以て表わした．また，α-アミラーゼの場合も同じく荻原[10]に

* 早稲田大学生物学教室 Biological Institute, Waseda University, Tokyo, Japan.

よる Wohlgemuth 法の改変法，すなわち，ヨード呈色による変量法を時間法に改変したものを用いた．

菌体の浸透価は前報のように，菌糸をスライドにとり，しょ糖液を注いで室温 15°, ×1200 で検鏡して求めた．

最近微生物による酵素の生合成機構を解明するための実験系として洗浄菌体が盛んに使用されている[11,12]．これによって菌体の生育増殖を防いで，菌体蛋白質の合成を起さないで，主として酵素の生合成だけを起させることができる．すなわち，上記の振盪フラスコ内に生成した黄白色球状の菌糸塊をブフナー・ロート上で吸引し，菌体を沪紙からはがし，多量の蒸留水または食塩水中に懸濁して十分攪拌洗浄し，いく度も沪別し，ネスレル試薬でアンモニウム反応がなくなるようにし，水分を測定してこれを二次培養に供した．

二次培養は 500 cc 振盪フラスコにぶどう糖 0.5 g と M/5 燐酸緩衝液 (pH 6.0) (M/5 KH_2PO_4, M/5 Na_2HPO_4) 50 cc を入れ，これに上の洗浄菌体 1 g を接種 30° で 20 時間振盪培養した．

実験結果

1．表面培養法によるプロティナーゼの生成と浸透価：表1は表面培養法によって2日毎に2個ずつのフラスコをとり出して調べた結果で，プロティナーゼの活性は2日目からすでにみられるが，4日目に急に増し，そのとき浸透価は 1.3 モルで，後までその値が続いた．プロティナーゼの活性度は6日目に最大となり，10 日目以後は急に低下し，菌体重量も少なくなっていった．この測定では，プロティナーゼの活性度はつぎの振盪培養よりも大きかった．

2．深部培養法によるプロティナーゼの生成と浸透価：表2は振盪培養法によって1日毎に2個ずつのフラスコをとり出して調べた結果で，浸透価は4日目に急に 1.2 モルにさがり，プロティナーゼが生成され，しだいに活性度は増大する．振盪培養では浸透価が 1.2 モル程度にさがらないとプロティナーゼは生成されないようで，このことは表3のように窒素の濃度を変えて浸透価を変動させると，浸透価の小さいもの程プロティナーゼが早く生成されることからもうかがわれる．

Table 1. Proteolytic activity, osmotic value and mycelial weight in *Aspergillus awamori* by the surface culture.

Days	2	4	6	8	10
Proteolytic activity	0.890	1.340	1.519	1.222	0.860
Osmotic value (M)*	1.5	1.3	1.3	1.3	1.3
Mycelial weight (g)	0.685	1.204	1.170	1.180	0.898

* in sucrose

Table 2. Proteolytic activity, osmotic value and mycelial weight in *Aspergillus awamori* by the submerged culture.

Days	1	2	3	4	5	6
Proteolytic activity	0	0	0	0.086	0.461	0.591
Osmotic value (M)	1.7	1.6	1.5	1.2	1.2	1.2
Mycelial weight (g)	0.110	0.550	0.990	1.120	1.190	1.250

Table 3. Proteolytic activity and osmotic value on the 3.5 th day in various concentration of NH_4NO_3 in *Aspergillus awamori* by the submerged culture.

Conc. of NH_4NO_3	Proteolytic activity	Osmotic value (M)
1.5 g/l	0.075	1.1
2.0 g/l	0.059	1.2
2.5 g/l	0.000	1.3

Table 4. Proteolytic activity and osmotic value in the washed mycelium of *Aspergillus awamori* by the submerged culture (20 hrs. incubation).

Days till washing after inoculation	1	2	3	4	5	6
Osmotic value before washing	1.7	1.6	1.5	1.2	1.2	1.2
Osmotic value after washing	1.0	1.0	1.0	1.0	1.0	1.0
Osmotic value after culture	1.1	1.1	1.1	1.1	1.1	1.1
Proteolytic activity	1.117	1.550	1.351	0.995	1.190	1.263

3. 洗浄菌体法によるプロティナーゼの生成と浸透価: 表4は一次培養をした菌体を1日毎に洗浄し，それを使って 30° で 20 時間二次培養した場合の結果を示す．洗浄後の浸透価は菌体の年令に無関係にすべて 1.0 モルにさがり，20 時間培養後には 1.1 モルになったが，2〜3 日目の洗浄菌体がプロティナーゼの生成が最も多いことが見出された．

この場合に浸透価とプロティナーゼ生成能との関係をさらに明らかにするために，接種してから4日後の菌体を 0, 1.0, 1.3, 1.5 モルの食塩水で洗浄したものを使って二次培養をしてみると，表5のような結果がえられた．すなわち，菌体の浸透価が 1.0 モルの場合にプロティナーゼの生成能が最も大きい．

Table 5. Proteolytic activity and osmotic value in *Aspergillus awamori*, when washed by the various concentration of NaCl solution on the 4th day after inoculation.

Conc. of NaCl (M)	0	1.0	1.3	1.5
Osmotic value after washing	1.0	1.0	1.3	1.5
Osmotic value after culture	1.1	1.1	1.1	1.1
Proteolytic activity	0.978	0.978	0.875	0.656

Table 6. Activity of α-amylase, osmotic value and mycelial weight in *Aspergillus awamori* in dextrin, sucrose and glucose by the surface culture.

Days		3	5	7	9
Dextrin	Activity	1.31	4.77	10.50	11.25
	Osmotic value (M)	1.4	1.3	1.1	1.0
	Mycelial weight (g)	0.054	0.095	0.080	0.125
Sucrose	Activity	0	0	0	0
	Osmotic value (M)	1.5	1.5	1.5	1.4
	Mycelial weight (g)	0.111	0.106	0.250	0.095
Glucose	Activity	0	0	0	0
	Osmotic value (M)	1.5	1.5	1.5	1.4
	Mycelial weight (g)	0.105	0 804	0.320	0.080

4. α-アミラーゼの生成と浸透価: 表6は Czapek 液のしょ糖を等量のぶどう糖，デキストリンに代えた培養基を用いた場合の結果で，デキストリンの場合にのみ α-アミラーゼが生成され，菌体重量はその場合に最小で，浸透価が 1.1 モルになった場合にアミラーゼの活性度が急増することが見出された．

考察および結論

上の実験結果によって．プロティナーゼとアミラーゼの生合成は菌体の浸透価に影響されることが知られる．酵素の生合成は細胞の内外の条件に応じて変化することは．葉のインベルターゼ，アミラーゼについて知られているが[3,4]，糸状菌の場合にも酵素の生合成は細胞の状態，とくに浸透圧に関係することが実証された．プロティナーゼとアミラーゼの生成の最適の浸透価は 1.1〜1.0 モルであるということができよう．この両酵素は糸状菌や細菌の菌体外生酵素のうちの代表的なもので，これらの酵素の生合成過程は菌体蛋白の生合成過程とは全く別個のものであるとされている[1]．そして，この両酵素の生成過程においては共通の酵素前駆体が存在するともいわれているが，最適浸透価が同じことからも，この両酵素は同じ原形質の状態において生成されるものと考えられる．

プロティナーゼの研究に使った No. 82 の菌株については，プロティナーゼの生成に対する炭素源の影響の結果は，従来多くの研究者によって報告されてきた細菌や糸状菌アミラーゼの生成に対する炭素源の影響の結果と多くの共通点のあることおよび培地に炭酸カルシウムを適量添加して培養中の培地の pH の低下を防げば最終の pH が大きくなるにしたがってプロティナーゼの活性度は減少するがアミラーゼの活性度は反対に増加することがわかっている[6]．さらに，林檎酸やエタノールを少量培地に添加することによって両者の活性度の割合は鋭敏に変化することも知られているので[6]，この両酵素の生合成には密接な関係があるものと考えられる．そして，この場合の最適浸透価はまた，前報で述べたようにクエン酸やグルコン酸醗酵のような有機酸醗酵の最適浸透価と同じことが注目される．

アミラーゼの実験において，培地に可溶性でんぷ

んを使った場合にもアミラーゼは生成され，活性度は正確には測りにくいがデキストリンにくらべては弱いが，しょ糖，ぶどう糖にくらべては大いに強く，3日後の浸透価はしょ糖，ぶどう糖のが1.5モルであるのに，でんぷんのは1.1モルと低く，アミラーゼはその頃から生成され始めた．

最後に，実験の便宜を与えられた早大応用化学科の武富教授，星野，宇佐美両氏に感謝する．

文　　献

1) Yoshida, F., J. Agr. Chem. Soc. Japan **28** : 80 (1954). 2) Crewther, W.G., and Lennox, F.G., Nature **165** : 680 (1950). 3) Yoshida, F., J. Agr. Chem. Soc. Japan **29** : 175 (1955). 4) Wetter, L.R., Can. J. Bot. **30** : 685 (1952). 5) Matsushima, K., J. Agr. Chem. Soc. Japan **29** : 87, 781 (1955). 6) Hoshino, J., Bull. Chem. Soc. Japan **31** : 884 (1958), **32** : 71 (1959). 7) Okazaki, H., J. Agr. Chem. Soc. Japan **29** : 181, 273 (1955). 8) Tonomura, K., and Tanabe, O., ibid. **30** : 511, 598 (1956). 9) Takami, W., Bot. Mag. Tokyo **70** : 140 (1957), **72** : 113 (1960). 10) Hagiwara, B., Ann. Rep. Fac. Sci. Osaka Univ. **2** : 35 (1954). 11) Nomura, M., Hosoda, J., Maruo, B., and Akabori, S., J. Biochem. Japan **43** : 251, 841 (1956), **44** : 87 (1957). 12) Fukumoto, J., Yamamoto, T., and Tsuru, D., J. Agr. Chem. Soc. Japan **31** : 421, 429, 506, 510, 545 (1957). 13) Okonenko, A., Nankowi sapiski, Kiew 456, 466 (1930). 14) Oparin, A., Erg. Enzymforshg. **III** : 62 (1934).

Summary

The present report deals with the relation between formation of proteinase or amylase and the osmotic value in *Aspergillus awamori*. Proteinase is scarcely formed before the osmotic value falls down to the critical value such as 1.3～1.2 M of sucrose. The critical osmotic value in the case of the surface culture is larger than that in the case of the submerged culture. In the latter culture, proteolytic activity can be measured only after the osmotic value decreases to 1.2 M. The osmotic value of the completely washed mycelium is 1.0 M and increases to 1.1 M after 20 hrs. incubation. But proteolytic activities in these cases differ as their ages as shown in the table. By washing the mycelium with NaCl solution of various concentrations, it was found that the optimum osmotic value for proteinase formation was 1.1～1.0 M and the same conclusion was valid for α-amylase formation.

Short Communication

Chihiro TAKAHASHI*: Anomalous Stomata on Polyploid Bracken

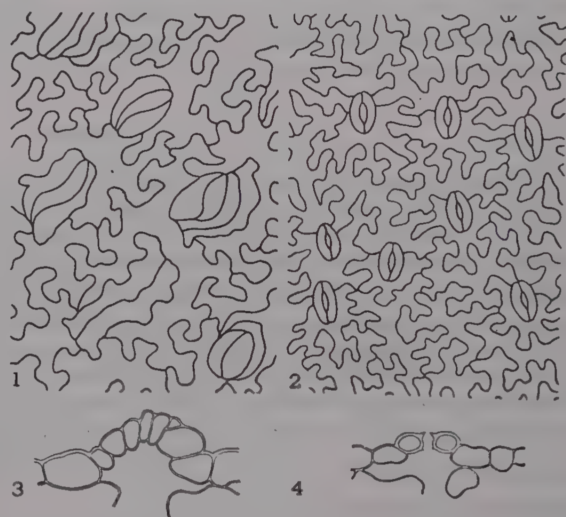
高橋千裕*: 倍数体ワラビの異状気孔

Received February 29, 1960

It was reported by the present writer that apospory could be induced in *Pteridium aquilinum* (L.) Kuhn var. *latiusculum* (Desv.) Und.[1]. He has got hundreds of polyploid brackens by means of aposporous regeneration.

It was revealed that there were many interesting features in polyploid brackens compared with normal diploid ones. Their stomata are larger than those of normal brackens but show the same structure, with the exception of three polyploid brackens which have extremely malformed stomata. There are many reports on multicellular or abnormal stomata of pathological or hybrid origin, or on those resulting from normal development[2,3,4]. But the unusual stoma reported here comes from polyploid origin and has unique features as follows: it consists of two to more than ten thin-walled cells having irregular forms instead of two thick-walled guard cells: it has no slit for gas exchange: not a stoma normal in structure and function is to be seen on these three polyploid brackens as far as the writer has observed.

Detailed report will be published later.



(1), (3) malformed stomata on polyploid bracken; (2), (4) normal stomata on diploid bracken; (1), (2) ×100; (3), (4) ×250.

References

1) 高橋千裕, 第20回植物学会大会講演 (1955). 2) Küster, E., Pathologische Pflanzenanatomie, 3 Aufl, Jena (1925). 3) Reuter, L., Protoplasmatologia Bd. 11-2, Wien (1955). 4) Fukasawa, H., Cytologia **23**: 128 (1958).

* Biological Laboratory, Department of General Education, Nagoya University, Nagoya, Japan. 名古屋大学教養部生物学教室

雑　　録

第9回国際植物学会に出席して

1959年8月19日から29日までカナダ国モントリオールで第9回国際植物学会がひらかれ，わたしは日本学術会議からの代表のひとりとして出席したので，すこしおくればせの感じもあるが，会のようすを報告しておきたい．

第8回の会は1954年8月にパリでひらかれたが，そのとき次回がモントリオールでひらかれるときまったのである．パリのまえは1950年にストックホルムでひらかれ，それまでは4年に1回のわりでおこなわれていた．いまどき5年に1回の国際学会というのは，あまりほかの学問にはないことで，学問の進歩に歩調をあわせるわけにはいかないとおもう．

今度の学会には14の部会ができ，ほかに今回から特別に Special Committee for Further Congresses という特別委員会がつくられ，またもうひとつ毎回もうけられる Resolution Committee もつくられた．わたしは6月にこの前者の委員を委嘱されていたので，前後4回おこなわれた会議のうち3回だけ出席した．委員はみな多用だったので，みな共通に出席できる時間を1時間なり2時間なりみつけることは，たいへんむずかしかった．この特別委員会はある意味ではかなり重要で，それは将来の，特に次回の会をどこでひらくかという問題と，どのような学会のやりかたをするかという問題とを討議したからである．この委員会の議長には K. V. Thimann 教授があたり，英独仏の3か国語を自由にはなして，会議の進行をなめらかにした．委員は P. Chouard (フランス)，A.L. Kursanov (ソ連)，E. Battaglia (イタリア)，A.A. Bitancourt (ブラジル)，H. Boyko (イスラエル)，Z. Cernohoraky (チェコスロヴァキア)，J.W. Groves (カナダ)，N. Hubbeling (オランダ)，E.O.G. Hultén (スウェーデン)，W. Rothmaler (東ドイツ)，J. Ch. Sengupta (インド)，J.-J.A. Symoens (ベルギー)，J. Walton (連合王国)，服部の15人であった．この委員会にでたはなしを要約すると，大体つぎのようになる．

次回開催地はベルギー国とする．この問題については，議長がパリの学会でベルギーから次回を自国でひらきたいという招待があったとのべたら，ベルギーの委員から，これについては，ベルギーの学者全部が相談をうけたわけではないので，国にかえったら，さっそく手紙でといあわせ，その結果を6か月以内に議長に通知するという発言があり，これはすぐ諒承された．ベルギーといっても，まずブリュッセルのほかには学会をひらけそうな都会はアントワープ以外にはないから，これで次回の国際学会はブリュッセルにきまったといってもよい．オランダの委員から，ベルギーは小さい国だし，オランダも小さい国なのだが，できれば援助をしたいという，もうしでがあり，つづいてドイツからも，おなじ趣旨の発言があった．

さて，開催の年であるが，ひろく植物に関連のあるコングレスをしらべてみると，1963年は非常にこんでいるので，今度も例外的に1964年にひらくことになった．

1964年のつぎはどこにするか，希望の国はないかという議長の発言にすぐこたえて，インドの委員から発言があり，正式の招待ではないが，インドの植物学者のよりよりの相談でインドでひらきたいという発言があり，委員のあいだから，インドの一番よい気候のときの12月から1月にかけては西欧諸国ではクリスマスがあり，年末年初をはずせばよいとか，8月という休暇のときはインドはまだ猛暑だからまずいだろうとか，いろいろな意見もでたが，よく研究のうえブリュッセルでの学会で正式の招待をしてもらいたいとの議長の発言できりとなった．わたしは，じつは，もし日本でひらいてもらいたいとの発言があるかもしれないとおもい，日本学術会議でしらべた結果，1964年までは日本で毎年2回ほど同会議のせわしなければならない国際学会があるが，それ以後はいまのところまだきまっていないということをたしかめ，またいく人かの植物学者と個人的にはなしあった結果，Congress は無理だが，Symposium もしくは Symposia ならやってやれないことはないだろうという漠然としたかんがえ

をもって会議にのぞんでいた．しかし，次次回がほぼインドでというようにみな理解したので，わたしからは積極的なはなしはなにももちださなかった．この委員会ではカナダの委員からきくと，モントリオールでの学会のためにあつめた金は 20 万カナダドルつまり 8 千万円だというから，とても日本ではできない相談だとおもう．

つぎに，学会のやりかたである．わたしは現在やっているような方式で学会を運営すること，つまり，たとえばこのモントリオールでのように 14 もの部会にわけ，広大な地域にちらばっている会場を右往左往したり，一講演 15 分ぐらいでは，まずまず人にあう，人のかおをみる，有名な人の講演をきくというぐらいがふつうで，ほんとうに学問を進歩させるということからは，すこしはなれすぎるのではないか，だからコングレスは 4 年か 5 年に 1 回でもよいから，そのあいだにシンポジアムを 2，3 まとめて 2 回か 3 回ぐらいひらいたほうがいいのではないかという意見をだした．委員のうちにも，みずからも，この意見をだそうとかんがえていた人もかなりあったようで，これは賛成をうけた．しかし，シンポジアムをあたらしくひらくには，この委員会のもち時間がわずかすぎるので，せめてブリュッセルでの学会では，講演時間を 30 分乃至 40 分（討論時間をもこめて）にしようというはなしがきまった．もちろん，この種のコングレスには世界中から学者があつまるので，かおみしりになるとか，会期中あるいは会がはててからのつきあいの端緒を得るとか，外国の研究室やそのほかの施設をみるとか，有用な面がおおいことも無視できないので，要は，このふたつの面の調節をどうするかが問題になるのであろう．

この委員会は，英独仏の三国語でしゃべるという原則で会議をやったのであるが，わたしは英語とドイツ語はしゃべれるが，フランス語ははやくちにはなされたのでは，意味がとれない．大事だとおもわれることは，議長に通訳をたのめるが，いつでも通訳をしてくれというわけにはいかない．このつぎブリュッセルでも，この委員がやはりつくられるとおもうので，また日本へも委員のわりあてがくるであろうし，だれが出席するようになるかわからないが，植物学研究連絡委員会でも，この点についてはかんがえる必要があろう．

この委員会のことはこのくらいにして，学会全体のことについてのべよう．出席者は 3240 名をすこしこえる程度である．もっとも登録しないで出席している人もいくらかみかけたし，実際の人数はもっとおおかろう．この数は最終日 28 日の午後登録票をかぞえた数だから，かなり正確にちかいとおもう．このうち日本人は 47 名でうち 6 名が夫人で学者ではない．それにしても 40 名以上という日本人が出席したというのは，おそらく破天荒といってもよいであろう．国がちかいということ，アメリカやカナダに滞在して勉強している人がヨーロッパよりもずっとおおいということが，この多数の原因だとおもう．かなり積極的にあちこちうごきまわっていた人があるのかとおもうと，リセプシャンの類で一度もかおをみせない人もあった．

14 の部会というのは．命名法，一般分類学および系統学，藻類学，菌類学（ここに地衣学，医学的菌類学のふたつの小部会が属している），植物病理学，蘚苔学，微生物学，形態学および解剖学，分類学および維管束植物の地理学，古植物学，生理学，生態学，細胞学および遺伝学，森林植物学である．

このほかダーウィンの「種の起原」出版 100 年記念，大会，部会などの招待講演がおこなわれ，カナダの科学映画，観光映画なども毎日上映された．会期の 2 週間まえぐらいからと会がおわってからの 1 週間ぐらい山岳地帯，森林地帯，海岸地帯，湿地などに顕花植物，シダ類，コケ類，藻類などを目的としたいろいろな遠足が 25 もよおされた．会議のあとの遠足にはオタワへ政府の研究施設を見学する遠足もあったし，会期中にはひとつだけセイント・ローレンス・シーウェイの見学の遠足があった．わたしは費用と時間との関係から，この遠足にしかくわわらなかった．婦人，こどものためにも，ladies' party，children party がひらかれ，またうけつけ，郵便局，銀行そのほかのかかりなども，まず普通以上に心をくばってあったと，わたしは感じたが，それでもこの学会の運営がまずいという批評を耳にした．

14 の部会の記事をかくなどということは，まず紙数にかぎりもあり，おもに生理学の部会にしかでていなかったので，とても不可能である．

29 日の朝 10 時から事務所のあるモントリオール高等学校の講堂で閉会式があり，ここで無事第 9 回国際植物学会はおわった．この席で，Thimann 氏の報告もあり，次回がブリュッセルで 1964 年にひらかれることに委員会で決定されたむねを報告し，参会者は拍手でこれを承認した． （服部静夫）

本　会　記　事

4月から役員に一部交代がありましたのでおしらせいたします.

幹　事

幹事長: 門司正三
庶務幹事: 清水　碩
会計幹事: 佐藤正一
図書幹事: 新津恒良
編集幹事: 吉田精一, 岩城英夫, 黒岩澄雄

編集委員

千葉保胤, 神谷宣郎, 太田行人, 原　寛, 門司正三, 下郡山正巳, 高宮　篤, 木村陽二郎, 八巻敏雄, 湯浅　明, 宝月欣二, 小野記彦, 大槻虎男, 小林義雄, 長尾昌之, 田川　隆, 林　孝三, 今村駿一郎, 新崎盛敏, 井上隆吉, 新家浪雄

新　入　会　員

(昭和 34 年6月〜昭和 35 年1月)

北海道支部

阿部　貞夫　北海道紋別市　鴻之舞高校
渋川　繁光　函館市八幡町　北海道学芸大学函館分校生物
松坂　聰　〃
武久　慎　北大理植
田中　滋郎　〃
庄　貞行　〃
大橋　裕　〃

東北支部

奥田　慎一　東北大理生物
鈴木　博　東北大川内分校生物
茂木　允彦　〃
大森　和彦　宮城県白石市調練場　白石女子高校
三井　英二　東北大理生物
三浦竹治郎　秋田市外旭川八柳
清水　大典　山形県米沢市　市立米沢郷土博物館
加藤　正名　山形市小白川町　山形大学文理学部生物
樫村　利道　福島市浜田町　福島大学学芸学部生物

関東支部

鈴木　隆光　江東区深川浜園町　農林省食料研究所
中村幸四郎　足立区南鹿浜町 23
小崎　道雄　世田谷区世田谷 4　東京農大
牛島　忠広　小金井市本町 6-1832 大沢方, 東京農工大繊維学部
村上　毅　〃
阿部　幸潁　静岡県三島市芝町　日大三島高校
宮口　柾子　足立区梅田町 1340, 王子中学
磯　三知子　南多摩郡稲城町大丸 922, 四谷第二中学
岡根　浩造　秋田県横手市住吉町　県立横手工業高校
小林　巌雄　東京教育大理地質鉱物
石川　照雄　北区西ケ原 3—35, 東京教育大理植
藤伊　正　世田谷新町 1—100, 東京教育大理植
横浜　康継　千葉県印旛郡印西町木下1500, 東京教育大理植
岡島　安隆　小金井市本町 1—1869　五味川方, 女子美術大附属高校
内田正之助　東京教育大理植
奥山　英虎　東京都八丈島八丈町大字中之郷
稲葉　二郎　新潟県西蒲原郡吉田町大字吉田中町今竹方
大場　達之　東京都世田谷区玉川奥沢町 3—277
渡辺　塔二　静岡県御殿場市永塚 59
岡安　広治　長野県上田市北天神町1824　県立須坂東高校
松下　登　府中市栄町　東京農工大一般教育部
村松　秋清　甲府市川田町 517　山梨県立蚕業試験場
比留間　実　神奈川県高座郡寒川町岡田 2600
北見　健彦　新潟県佐渡郡相川町　新潟大学附属臨海実験所
大西　一博　都立大理生物
大隈　正子　文京区高田豊川町18　日本女子大生物学教室

中部地方

榛村　恵夫　静岡大教育生物
渡辺　昌彦　松本市県町　信州大文理学部
牛山　六男　岐阜県岐阜市外鄒加町　岐阜大農学部
内藤　雅子　浜松市下池町 155　信愛学園
吉田　和典　名古屋市南霞町　市立桜台高校

近畿支部

花房　尚史　阪大理植物

清水　晃　阪大理植物
小川　幸持　京大農応用植物
北川　尚史　京大理植物
佐藤　治雄　大阪市大理生物
岩槻　邦男　京大理植物
山本　武　大阪市大理生物
徳田　真一　〃
江越千代子　神戸市東灘区住吉町唐松 817
矢内　正弘　兵庫県姫路市八代中町 599

中国・四国支部

関　太郎　広島大理植
根平　邦人　〃
上村　亨　〃
中西　稔　〃
江口　亨　山口市河原　山口大文理生物
吉村　庸　高知市朝倉乙 775　高知学芸高校
青木　充之　島根県簸川郡斐川村出西 2389

九州支部

奥　達夫　鹿児島大文理生物
松島　幹夫　熊本県熊本市神水町 200　農林省蚕糸試験場九州支場
大内　準　福岡県田川郡香春町　県立田川高校

外国会員

金　宇鎬　韓国江原道春川市孝子洞　春川農科大学獣医科

住所変更

(34 年 6 月より 12 月末日まで)

東北支部

菅原　繁蔵　山形県北村山郡東根市神町

関東支部

青山　俊吉　足利市有楽町足利女子高校内
野村　清　北区田端町 271　金墻方
飯島　敏雄　長野県大町市大町中学校
宮地数千木　横須賀市清泉女子大学
椿　啓介　世田谷区三宿町 381–21 長尾研究所
曾根田正己　〃
木下　哲雄　長野県松本市蟻ケ崎北区 553–43
館岡　孝　杉並区方南町 543
安村　明　板橋区蓮根町 2–10 蓮根公団住宅 3041
丹田誠之助　世田谷区世田谷 4–413 安田方
米田　芳秋　三島市谷田 国立遺伝研
長谷川　昇　埼玉県川口市芝神戸 2798
萩原　時二　横浜市神奈川区栄町 3–48
尾崎　清　新宿区諏訪町 136–215
小宮　定志　千代田区富士見町 1–3 日本歯科大学生物
小嶹　吉之　川崎市堀川町 580 明治製菓研究所
会沢　正義　横浜市磯子区杉田町 1712 横浜市立浜中学校
浅井　康宏　千代田区神田三崎町 1–7　東京歯科大学保存学教室
佐藤　茂　新潟市下所島字向山 50–3
江本　義数　世田谷区祖師ケ谷 1–1068

中部支部

坂本　充　名大理 水質研究室
堀米　和雄　長野県鉄道管理局飯山車掌支区

北陸支部

三上　寿一　愛知県中島郡祖父江町上牧　祖父江中学校

近畿支部

杉山　弘幸　枚方市大字渚 240–6
岩田　修造　神戸市灘区六甲台六甲住宅 10–506

中国四国支部

国沢　鎮雄　高知県香美郡土佐山田町　山田高校
高橋　節　〃
日出　武敏　鳴門市撫養町南浜鳴門第一中学校
太刀掛　優　呉市広町塩焼北町
竹下　政範　広島県佐伯郡吉和村　津田高校吉和分校

九州支部

末宗　正明　大分県中津市如水区是則　東中津中学

名誉会員　Prof. Dr. W. Ruhland 氏は本年 1 月 9 日肺と心臓との疾患でおなくなりになりました．ふかく哀悼の意を表します．

日 本 植 物 学 会

Ecological and Physiological Studies on the Vegetation of Mt. Shimagare
V. Intraspecific Competition and Productivity Difference among Tree Classes in the *Abies* Stand*

by Sumio KUROIWA**

Received September 2, 1959

Intraspecific competition in a plant stand, which reflects the productivity difference between constituent plants, must be studied by means of the analysis based on dry matter production of the interaction between the physiological functions and the microenvironmental factors. A few studies on this problem have been carried out from the viewpoint of dry matter production, e.g. in an ash forest by Boysen Jensen[1]) and in an aspen forest by Satoo *et al*[2,3]).

The intraspecific competition has apparently a close relationship with the characteristic growth pattern of the subalpine coniferous forest on Mt. Shimagare, which consists of several forest units running along the contour of two *Abies* species, *Mariesii* and *Veitchii*[4]). In previous papers[5,6]), as to this problem, the author has already discussed the frequency distribution of tree size, productive structure of the forest stand, and furthermore photosynthesis, respiration and nitrogen content of the needles and branches, in regard to needle age, tree size class and tree species.

In the present paper, the difference in annual productivity among tree classes will be demonstrated on the basis of light distribution in stand and physiological functions concerned with dry matter production, especially in a 20-year-old *Abies* stand of Forest Unit V.

Estimation of annual net production

Annual net production of a tree is the sum total of annual dry weight increments in leaves, branches, trunk and roots. These increments were estimated in the *Abies* trees from annual change of the measures characteristic of each tree size class, as follows.

The annual needle increment consists of the total dry weight of newly formed needles (F_1) and the annual dry matter increment in the older needles. The product of the number of new needles (n_1) and their mean dry weight (f_1) after development makes the former. And the latter can be obtained from the total number of older needles (N) and their mean annual dry weight increment (α) which was nearly of constant rate throughout the life from 1 to 9 years of needles. The needle number of the same age decreased in an *Abies* tree at exponential rate (λ) as the age (t) increased (see Fig. 1), so that N could be expressed as a function of λ and n_1. These measures, n_1, f_1, α and λ, characteristic of each tree class are summarized in Tab. 1. With these characteristics, the annual increment of a tree in needle dry weight (ΔF) can be calculated in each tree class by using the following formula, the maximum

* Supported by the Grant in Aid for Scientific Research from the Ministry of Education.
** Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

needle longevity being assessed as 8 years (cf. Fig. 1).

$$\Delta F=F_1+\alpha N=f_1n_1+\alpha\sum_{t=2}^{9}n_1e^{-\lambda(t-1)}\fallingdotseq f_1n_1+\alpha n_1\int_{1.5}^{9.5}e^{-\lambda(t-1)}dt \quad\cdots\cdots\cdots\cdots (1)$$

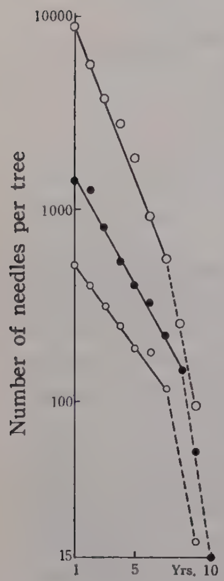


Fig. 1. Relation between number of needles and their age, in each of *Abies Mariesii* trees 20 years old belonging to three tree classes. Upper line, dominant tree; middle, intermediate; lower, suppressed.

A large branch is composed of several branchlet groups each of which consists of branchlets covered with needles of the same age. In each tree class, it was empirically determined that both the mean branchlet radius (r) (measured at its middle portion) and the mean branchlet length per needle (l) in each branchlet group were expressed with a linear function concerning age respectively, i.e., $r=r_0+at$ and $l=l_0+bt$, where r_0, a, l_0 and b were constants characteristic of each tree class (cf. Tab. 1). In the above-mentioned calculation of ΔF, the needle number per tree was formulated as a function of t, n_1 and λ. Also the total branchlet length in a branchlet group can therefore be expressed as a function of these three values, together with l_0 and b. Combining this function with the equation $r=r_0+at$, one will obtain the annual dry matter increment in each branchlet group, and consequently the total annual increment in dry matter of the branch system of a tree (ΔC_B). The latter can be estimated according to the following formula, without measuring the branchlet length etc. respectively when the number of new needles is known.

$$\Delta C_B=\eta\pi n_1\left\{r_0^2l_0+2a\int_{1.5}^{9.5}[r_0+a(t-1)][l_0+b(t-1)]e^{-\lambda(t-1)}dt\right\} \quad\cdots\cdots (2)$$

where η is bulk weight (g. dry matter in cubic cm.) of branchlets —— the measured values were in the range of 0.3–0.5, mostly 0.45 g./cm³. The annual increment in the branchlets more than 10 years old was neglected as their number being small enough to be neglected.

The trunk dry weight (C_H) is able to be estimated by the formula $C_H=0.67\pi\eta'(D/2)^2\ H$, where D is the basal diameter, H, the height, and λ', bulk weight (0.48 g./cm.³) of trunk—0.67 is the form factor determined at the *Abies* stand concerned. The difference between the C_H in the current year and that in the preceding year, which were calculated with the formula on the basis of the relation between tree size and its annual increment (Fig. 2), corresponds to the annual increment of the trunk in dry weight in the latest year. The annual

Table 1. Determined values of the constants α, λ, n_1, f_1 in Formula (1), and r_0, l_0, a, b in Formula (2), with regard to needles and branchlets of *Abies Mariesii* trees in four representative classes. A 20-year-old *Abies* stand in Forest Unit V.

Tree class	α (mg.)	λ	n_1	f_1 (mg.)	r_0 (cm.)	l_0 (cm.)	a (cm.)	b (cm.)
Dominant	0.20	0.42	9000	2.5	0.115	0.06	0.00700	0.024
Codominant	0.22	0.37	4000	2.1	0.090	0.07	0.00725	0.025
Intermediate	0.24	0.31	1400	1.7	0.070	0.09	0.00750	0.026
Suppressed	0.30	0.17	100	1.2	0.045	0.25	0.00800	0.028

dry matter increment in the root system was assumed as one fifth of that in the aerial part, after Ovington's data[7]) in *Pinus sylvestris*. The sum total of these annual increments in every tree organ (needles, branches, trunk and roots) makes the net dry matter production per tree; the values obtained in a 20-year-old *Abies* stand of Forest Unit V are shown in relation to the tree dry weight class in Fig. 3 and Tab. 2. The dominant tree has clearly the highest values of the tree classes not only in net production but also in relative productivity (g. net production/g. tree dry weight) and net assimilation rate (g. net production/g. needle dry weight). Moreover, the dominant had also the highest distribution ratio for leaves (percentage of $\Delta F/\Delta W$)[8]) (dominant : codominant : intermediate : suppressed=42 : 40 : 37 : 26%).

According to Boysen Jensen's data[1]) in an unmanaged *Fraxinus* stand of 12 years old —— a modi-

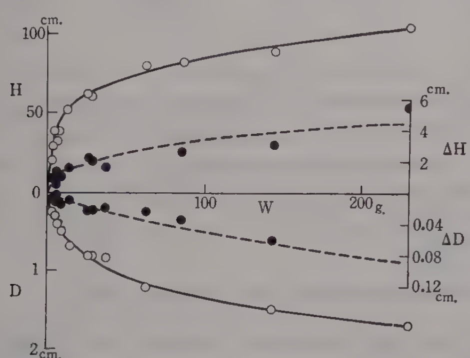


Fig. 2. Trunk height (H), its annual increment (ΔH), basal diameter (D) and its annual increment (ΔD), related to tree dry weight (W). *Abies Mariesii* in a 20-year-old *Abies* stand (Forest Unit V).

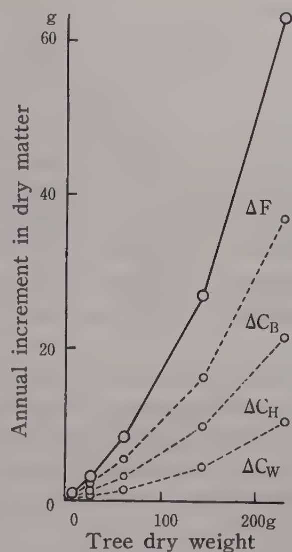


Fig. 3. Relation of the annual dry matter increments in needles (F), branches (C_B), trunk (C_H) and roots (C_W) to tree dry weight. *Abies Mariesii* in a 20-year-old *Abies* stand (Forest Unit V). The sum total of the increments makes the net production of a tree.

Table 2. Dry weights in g. of tree, wood and needles, and annual increment (net production) in g. d.w. of individual *Abies Mariesii* trees. Relative productivity (4)/(1), annual increment per wood (4)/(2) and net assimilation rate (4)/(3) are also shown. A 20-year-old *Abies* stand (Forest Unit V).

Tree class	1. Tree dry weight	2. Wood dry weight	3. Needles dry weight	4. Annual increment	5. Relative productivity	6. Annual increment per wood	7. Net assimilation rate
Dominant	230	129	62.5	63	0.27	0.49	1.01
Codominant	142	85	31.0	27	0.19	0.32	0.87
Intermediate	60	37.5	12.5	8.4	0.14	0.22	0.67
Suppressed	8	5.0	1.5	0.92	0.11	0.18	0.61

fication concerning the top weight by the present author are shown in Tab. 3 ——, the smaller the tree size class, the lower was the ratio of annual net production to the total wood (col. 6), and the higher was the ratio of the dry matter loss by

branch fall to the annual net production (col. 7). In other words, in the suppressed trees the small wood increment should result not only from the lower net production but also from the relatively higher loss by branch fall. This may suggest that the longevity by any tree organ is closely dependent on the relative productivity. Similarly, in the *Abies* trees the larger the tree weight, the higher was the ratio of

Table 3. Ratio of annual dry matter increment to wood weight, and ratio of fallen dead branches to the former, in a *Fraxinus* stand 12 years old observed by Boysen Jensen[1]). Measuring unit is ton dry matter/ha. The figures in parentheses are relative values.

Tree class	1. Wood	2. Annual increment in wood	3. Fallen leaves	4. Fallen dead branches	5. Net production (2+3+4)	6. P_n per wood (5/1)	7. B_d per P_n (4/5)
Dominant	13.4	2.64	1.69	0.32	4.65	0.347 (54)	0.069 (10)
Intermediate	9.7	1.38	0.78	0.17	2.33	0.240 (37)	0.073 (11)
Suppressed	6.2	0.07	0.25	0.08	0.40	0.064 (1)	0.200 (29)

dry matter increment to the wood weight as well as to the total tree weight (Tab. 2). From these facts the following may be deduced: (1) The ratio of the dry matter loss by branch and needle falls to the net production increases with reduction of tree size. (2) The relative growth rate (g. dry weight of tree growth/g. tree dry weight) diminishes with lowering of the tree size classes at a greater rate than the dry matter production does.

Analysis of productivity difference among tree size classes

The amount of net production (ΔW) is the difference between the amount of photosynthate and the total respiratory loss, i.e. $\Delta W=F(A-R)-CR_c$ (Iwaki[8,9]); F, A and R indicate respectively the total dry weight, photosynthetic and respiratory rate of photosynthetic system (needles), while C and R_c indicate the total dry weight and respiratory rate of non-photosynthetic system (trunk, branches and roots). The relative productivity (P_r) mentioned before, i.e. $\Delta W/W$ (W: total tree dry weight), is expressed with the formula $P_r=F(A-R)/W-CR_c/W$. Comparing the tree weight classes in each term of this formula on the basis of the relative weight of each organ in Fig. 4 and the physiological data in a previous paper[6]), (1) both F/W and $A-R$ decrease with lowering gradation in the tree weight classes, but (2) the value CR_c/W seems rather invariable with the tree weight class, since C/W increases but R_c decreases with the decrease in tree weight. In conclusion, even under the same microenvironmental conditions, the larger tree in size and weight can possess higher relative productivity than does the smaller tree.

Out of numberless environmental factors, light and temperature must be considered first of all, because the former directly affects photosynthesis, and the latter decides the rate of photosynthesis as well as of respiration. The productive structure of *Abies* stands and the light distribution in them, the most important factors in matter production, have already been illustrated in a previous paper[5]). —— The light intensity decreased exponentially towards the stand base mainly by means of shading of needles. The tree which was lighter in weight was generally lower in its tree height as well as in its canopy height. Judging from these facts, the smaller trees should receive with their canopies weaker illumination than the larger trees do. This

is also brought to light with Fig. 5. About thirty light measurements were carried out at random at each stratum and the mean light intensity was obtained, in a 20-year-old *Abies* stand (Forest Unit IV) of 100 cm. high. The illumination received by the suppressed was only 3–13 percent of full light which was prevailing on the dominant. The light intensities measured just above the crowns of eighty small trees were, in general, slightly higher than the mean value at the same stratum; the surviving small trees grew at brighter places.

The temperature difference between the forest strata is not so large as observed in light factor. Even in a 60-year-old *Abies* stand of 7.5 m. high, for example, the highest temperature of 6.5° was measured just below the top of crown, and the lowest of 4.0°, above the forest floor (2 p.m., May 7, 1959—slightly cloudy). The temperature in various organs of the *Abies* trees may not much differ from the temperature of surrounding air, judging from a study of the diurnal temperature course in buckwheat stands[10]). Accordingly, a great difference in the canopy height seems to bring about only a little difference in the mean daily temperature of tree organs among tree size classes, and such a temperature difference can cause a slight difference in productivity among them. The principal cause responsible for the productivity difference among tree classes, however, must be the above-mentioned clear difference in illumination received by each tree class.

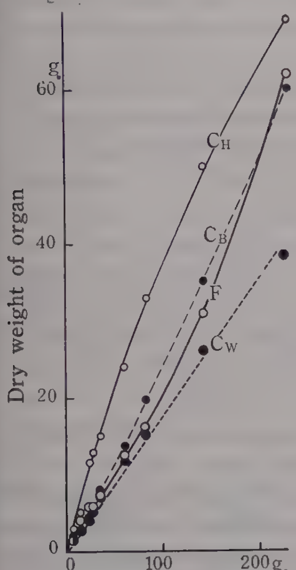


Fig. 4. Relation between tree dry weight and dry weight of each organ, in *Abies Mariesii* 20 years old (Forest Unit V). Signs mean the same in Fig. 3.

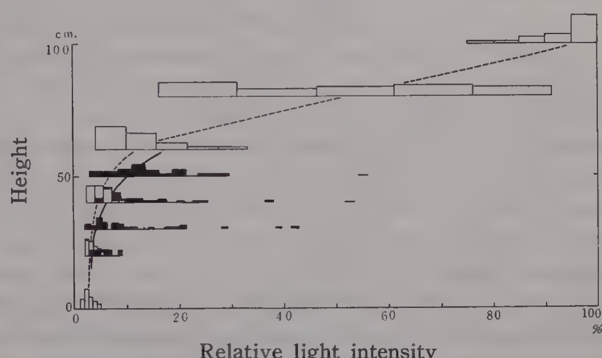


Fig. 5. Frequency histograms of relative light intensities within an *Abies* stand of 20 years old (Forest Unit IV). Blank polygons and broken line indicate the light intensities measured at random at each stratum and the vertical distribution of their mean values. Solid polygons and line indicate the light intensities measured just above each canopy of intermediate and suppressed trees and their mean values. On August 18, 1958 (cloudy).

Resynthesis of different productivity in tree classes

The important role of light factor in growth competition, which was inferred in the preceding section, should be proved by resynthesis of dry matter production of the tree, with combining the productive functions and microenvironment factors, especially light factor, characteristic of each tree class. This was carried out as to the same *Abies* stand in which the annual net production has directly been estimated

(p. 165) (20 years old, Forest Unit V—cf. also Fig. 6 in a previous paper[5])). Net production rate was determined in this section as the difference between assimilate and dissimilate calculated from photosynthetic and respiratory activity of unit plant tissues[6]), the former of 3-year-old needles being used in each tree class as an average for all of the needles of various ages.

Recently, Bourdeau[11]) has found that the net photosynthesis of conifers kept outdoors decreased to zero or sometimes even negative in the cold season with severe frost. Pisek and Winkler[12]) have also observed a similar depression of photosynthetic rate in subalpine conifers of the Central Alps (at Patscherkofel, 1840 m. above sea level), when daily minimum temperatures were below −15°. The daily minimum temperatures in Mt. Shimagare (Tab. 4) indicate that a similar cold season, during

Table 4. Temperature condition at Mt. Shimagare. Mean temperatures are the averages of temperatures at the highest and the lowest altitudes on Mt. Shimagare[4]) Minimum and 10 a.m. temperatures were assessed from the data observed at the Yatsugatake Experimental Farm (1920 m. above sea level), 10 km. southwards from Mt. Shimagare.

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Mean temp.	−13.6	−14.1	− 9.5	−2.1	3.1	8.8	11.6	13.7	9.0	4.2	−1.8	−12.3
Minimum temp.	−18.9	−19.6	−14.9	−7.3	−1.9	4.2	7.2	9.5	4.5	−0.6	−6.8	−17.5
Temp. at 10 a.m.				0.4	5.4	10.3	13.7	15.9	11.2	6.4	0.7	

which no photosynthate can be expected, extends from the beginning of December to the end of March.

In order to obtain the annual (or strictly of vegetative season) production per tree in each tree class, the daily gross production should be calculated at first, and then the monthly one. Symmetric diurnal illumination curve with a midday maximum, 150, 70 and 20 kilolux in a clear, a slightly cloudy and a rainy cloudy day respectively, was adopted according to the photoelectric observations at the site. The monthly number of these days was estimated to be 1, 8, and 21 days respectively on the basis of meteorological data at the montane observatories around Mt. Shimagare.

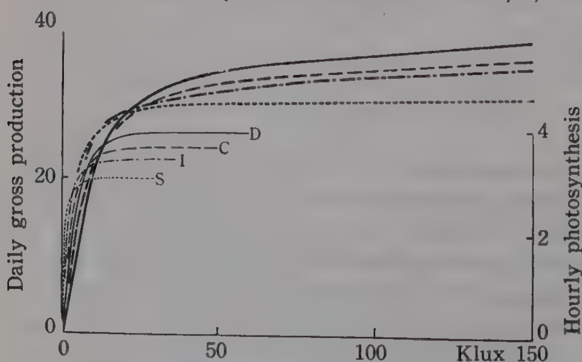


Fig. 6. Hourly light-photosynthesis curves (thin ones), and daily gross production curves (thick ones) related to daily maximum illumination intensity. In 3-year-old needles of *Abies Mariesii* (20 years old). Both ordinates are scaled in mg. dry matter/g. needle dry weight, at 20°. D, dominant; C, codominant; I, intermediate; S, suppressed. In summer, 1958.

The daily gross production per g. dry weight of needles (at 20°) was illustrated in Fig. 6 with coordinate of daily maximum illumination, by means of combining the diurnal course of illumination and the light-gross photosynthesis curve at 20° (cf. Monsi and Saeki[13]), Davidson and Philip[14]), Saeki[15])). The photosynthesis of needles in each stratum of the stand was calculated in consideration of the light intensity received by them. The absolute light intensity was calculated from the relative value as follows: (1) Relative light intensity at each height

of needles was determined from a vertical distribution curve of illumination in the stand (Fig. 6 of a previous paper[5])). (2) This value was multiplied by factors of 1.4, 1.2 and 1.1 for the suppressed, intermediate and codominant respectively, because these trees survive, as seen in Fig. 5, in the spots somewhat brighter than the mean light intensity. (3) The relative intensities of light received by needles were obtained by multiplication of extinction coefficient to the above-mentioned light intensities (cf. Saeki[15])); the extinction coefficient was evaluated as 0.75 from the minimal light intensity of 3 percent and the leaf area index of 5 in the stand[5]). (4) These relative light intensities were converted into absolute ones by multiplying absolute illumination according to the weather conditions.

With combining these absolute light intensities, the light-daily gross production curves in Fig. 6, and the dry weight of needles in the stratum, the daily photosynthate in each stratum was easily calculated, and the sum total of the latter gave rise to the daily gross production of a tree. These procedures were made for each weather condition and for each tree weight class. Monthly gross production per tree was computed, in each class, from the daily gross production in consideration of the number of clear, cloudy and rainy cloudy days in the month.

Then, in regard to the seasonal change of productivity the temperature factor becomes cardinal factor to be considered. Here, the author simply multiplied, with an assumption that photosynthesis at a low temperature will be depressed at the same rate in the whole range of illumination, the monthly gross production calculated above by the ratio of the light-saturated photosynthesis for the monthly mean temperature at 10 a.m. (Tab. 4) to that for the optimum temperature of 20°; these photosynthetic values were determined in the photosynthesis curve in Fig. 1 of a previous paper[6]). The monthly gross productions per tree thus obtained in each tree class for the vegetative season (from April to November) are illustrated in Fig. 7. The integration of these monthly productions gives the annual gross production of a plant in each class (the uppermost line in Fig. 8). It is quite clear that the annual gross production decreases remarkably with lowering the gradation in the tree classes (cf. also Tab. 5).

The annual dry matter loss by respiration was calculated by the same method as in the analyses by Boysen Jensen[1]), Iwaki[8,9]) and Hogetsu *et al*[16]), through combination of temperature effect on respiration and monthly mean temperature (Tab. 4). The results are given in Fig. 7 and Tab. 5. The annual net production is, as shown in Fig. 8, the surplus of the annual gross production above the annual total loss by respiration of the tree. The difference among the tree classes was severer in net production than in tree dry weight and gross production (Tab. 5).

The gross and net production of the 20-year-old *Abies* stand whose standing crop was ca. 2.0 kg./sq.m. (cf. Fig. 6 in a previous paper[5])) were respectively 1.1 and 0.5 kg./sq.m., being calculated with the value of gross and net production of each class tree and the number of trees in each class (Tab. 5). The annual growth calculated from the standing crop difference between the 20-year-old stand and the 23-year-old one (2.6 kg./sq.m.—in the said Fig.) was about 0.2 kg./sq.m. The gap between the net production and the annual growth amount may be filled with the dead trees and fallen needles and branches. On the other hand, these rather too small values appear to result from the low temperature in the habitat and the slow growth of the *Abies* trees in juvenile stage.

These calculated values were more or less the same as the results directly obtained by the analysis of tree size measures already discussed. The trend of decrease

in the relative values of dry matter production with the tree size diminution was quite in parallel —— the relative values of the net production in the previous section (p. 165) were 100:43:13:1.5, —— although the net production calculated from photosynthesis was somewhat higher in absolute value than the directly estimated. The

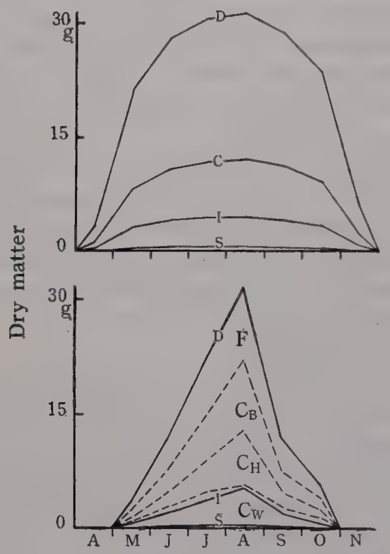


Fig. 7. Seasonal changes of monthly gross production (upper) and monthly loss by respiration (lower), in *Abies Mariesii* trees of a 20-year-old stand (Forest Unit V). In the respiration of the dominant, the components for each organ are also illustrated. Signs are the same as in Figs. 3 and 6.

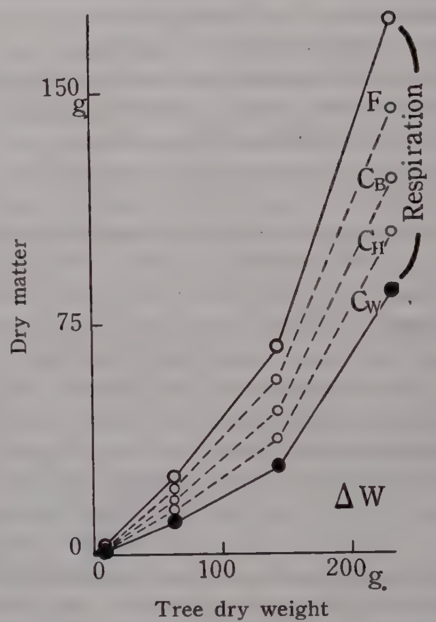


Fig. 8. Relation of annual gross production (upper solid line), annual respiration loss by each organ, and annual net production (ΔW), to tree dry weight. In *Abies Mariesii* trees of 20 years old (Forest Unit V). Signs indicate the same as in Fig. 3.

Table 5. Calculated gross and net productions of individual *Abies Mariesii* trees in dry matter (g.), and ratio of gross production to tree weight and of respiratory loss to gross production (g./g.). The same 20-year-old *Abies* stand in Table 2. The figures in parentheses indicate the relative values.

Tree class	Tree weight (W)	No. of trees	Gross production (P_g)	Respiratory loss (R_t)	Net production (P_n)	P_g/W	R_t/R_g
Dominant	230 (100)	3	173 (100)	86.6	86.4 (100)	0.75	0.50
Codominant	142 (62)	5	70 (40)	40.0	30.0 (35)	0.49	0.57
Intermediate	60 (26)	8	24 (14)	14.3	9.7 (11)	0.40	0.59
Suppressed	8 (3.5)	20	2.5 (1.4)	1.5	1.0 (1.1)	0.31	0.60

temperature-photosynthesis curves of broad-leaved evergreen trees[17]) have revealed the fact that the photosynthetic activity of leaves was generally lower in winter even at the same experimental temperature, despite of lowering of the optimum temperature for photosynthesis. Therefore, the value based on the summer photosynthesis

curves may, resulting in an overestimation, be accountable for the said difference between both net productions.

The general agreement between the values calculated in different ways supports the mentioned deduction that the intensity of illumination received by the crown mainly gives rise to the productivity difference among tree size classes. The final decision in intraspecific competition must result from the productivity difference. The ratio of gross production (P_g) to tree weight (W) demonstrates a productive efficiency of the tree dependent on light factor, and the ratio of the total respiration (R_t) to P_g does a respiratory loss rate mainly dependent on C/F-ratio[8,9]), where C and F are respectively the dry weights of non-photosynthetic and photosynthetic system. As the net production (P_n) is $P_g - R_t$ and P_n/P_g is $1-R_t/P_g$, the relative productivity (P_n/W) can be analysed as $(P_g/W)\times(P_n/P_g)$ or $(P_g/W)\times(1-R_t/P_g)$. This means that, if P_g/W is larger and R_t/P_g is smaller, the relative productivity should become higher. Therefore the dominant which bears these features (cf. Tab. 5) can perform the highest gross production with high efficiency of leaves under high illumination, and consumes rather small portion of the photosynthate in respiration to maintain itself. So growth of the dominant becomes extremely larger than that of the others with the lapse of time, bringing about further tree height difference and consequently large difference in light factor among them. And the reverse is the fact in the suppressed. The intraspecific competition which appears in the growth difference should be severer in the stand with wider frequency distribution in plant weight.

Summary

Intraspecific competition for weight growth was analysed in constituent *Abies* trees within a 20-year-old *A. Mariesii* and *A. Veitchii* mixed stand of Forest Unit V on Mt. Shimagare.

1. Annual dry matter increments in needles, branches, trunk and roots of each of trees, which were classified into the dominant, codominant, intermediate and suppressed, were estimated by the mathematical analyses of tree measures. Annual net production, the sum total of these increments per tree, increased with rise in gradation of the tree weight class. Also the relative productivity (net production/tree weight), the net assimilation rate and the relative growth rate indicated the same trend.

2. The inner and outer factors concerned with the dry matter production were discussed. The somewhat high proportion of needles for the total dry weight and the high light intensity received by needles were responsible for the high productivity in the dominant class.

3. Annual gross production per tree was calculated in each tree weight class by combining photosynthetic activity and light factor received by each canopy, with correction by seasonal temperature changes. Annual respiration loss was also calculated.

4. The difference between the annual gross production and the annual respiration loss gave the net production per tree. This value was in accord with the annual net production determined by the analyses of tree measures, in each class. The accordance may prove the soundness of the calculations.

5. The suppressed tree made relatively small gross production under weak illumination, but high respiration loss comparing with its dry weight. So the growth rate of the suppressed became very low. The reverse was seen in the dominant.

Such difference in matter production between the dominant and other tree classes should cause marked growth difference with the lapse of time.

The author's grateful acknowledgment is expressed to Prof. M. Monsi, Prof. K. Hogetsu and Assistant Prof. T. Satoo for their guidance and suggestions under which this work was accomplished.

References

1) Boysen Jensen, P., Die Stoffproduktion der Pflanzen, Jena (1932). 2) Satoo, T., Nakamura, K., and Senda, M., Bull. Tokyo Univ. Forest. No. 48, 65 (1955). 3) ——, Kunugi, R., and Kumekawa, A., ibid., No. 52, 33 (1956). 4) Oshima, Y., Kimura, M., Iwaki, H., and Kuroiwa, S., Bot. Mag. Tokyo **71**: 289 (1958). 5) Kuroiwa, S., ibid. **72**: 413 (1959). 6) ——, ibid. **73**: 133 (1960). 7) Ovington, J. D., Ann. Bot. **21**: 287 (1957). 8) Iwaki, H., Jap. Jour. Bot. **16**: 210 (1958). 9) ——, ibid. **17**: 120 (1959). 10) Kuroiwa, S., and Monsi, M., Jour. Agr. Meteor. **12**: 41 (1956). 11) Bourdeau, P. F., Ecol. **40**: 63 (1959). 12) Pisek, A., und Winkler, E., Planta **53**: 532 (1959). 13) Monsi, M., und Saeki, T., Jap. Jour. Bot. **14**: 22 (1953). 14) Davidson, J. L., and Philip, J. R., Climatology and Microclimatology (p. 181), UNESCO (1958). 15) Saeki, T., Bot. Mag. Tokyo **73**: 55 (1960). 16) Hogetsu, K., Oshima, Y., Midorikawa, B., Sakamoto, M., Tezuka, Y., Mototani, I., and Kimura, M., Jap. Jour. Bot. (in press). 17) Kusumoto, T., Jap. Jour. Ecol. **7**: 126 (1957).

摘　　要

縞枯山の植生についての生態学的ならびに生理学的研究
V. 種内競争による階級間の物質生産の差異について

黒　岩　澄　雄

すでに報告された縞枯山第 5 *Abies* 森林に属する 20 年生林分における同種間競争を前報の物質生産機能や群落構造[5,6]を用いて物質生産の立場から解析した．

葉，枝，幹，根における乾量の年間増分の総量を個体当り年純生産量とした．葉における増分は葉令増加にともなう一枚当り葉重増加量と葉数とから求め，枝における増分は枝令増加による枝直径増加量と枝容積当り乾物重とから求め，幹における増分は年輪増加量と幹容積当り乾物重とから求め，また根における増分は地上部重増分の 1/5 とした[7]．年純生産量の個体重に対する比も個体当り葉重に対する比も階級木の増大にともない増加した．*Fraxinus* についてのデータ[1]を解析した結果，生産力の低い小個体ほど落枝率（落枝量/純生産量）は大きかったので生長率はさらに小さくなると推論された．

大個体になるほど同化器官重の個体重に対する割合は大きく，非同化器官重のそれは小さかった，また，大個体は小個体よりも同化率（呼吸率もいくらか）が大きかったので，物質生産の面で大個体ほど有利といえる．環境条件のうち，温度では大差なかったが，光では大個体ほど樹冠が高く明るいところにあった．したがって，階級間における生産力の差異は同化器官の割合，生産機能および光要因の差異によると推定された．以上の推論をたしかめるため，各個体の純生産量を年総物質生産量と年物質消費量の差として計算した．生産量は樹冠の受光量と同化率とから月気温を考慮して計算し，消費量は各器官の呼吸率と月気温とから計算した．この値は樹幹解析にもとづいて得られた純生産量より少し大きかったが，階級木の増大にともない急激に増加するという傾向では全く一致していた．このことは種内競争における光要因の重要性をたしかにするものである．（東大理学部植物学教室）

Studies on the Light Controlling Flower Initiation of *Pharbitis Nil*. VI. Effect of Natural Twilight

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

Received September 9, 1959

Many investigators consider that the photoperiod affecting flower initiation begins at the time at which luminosity becomes 1–10 lux and ends at the time at which luminosity is also 1–10 lux. This conclusion is based upon the fact that the light of this intensity inhibits the dark process of photoperiodic induction, if the light of this intensity is used as a source of an illumination for a light-break of the dark process or as a supplementary light[1,2,3]). In *Pharbitis Nil*, too, plants subjected to light of 1–10 lux for 16 hours or more can not initiate a flower primordium, and those subjected to complete darkness for 16 hours or more can do so readily[4,5,6]). But as has been reported previously, the first process of the inductive dark period is believed to be a relatively light-stable one and can proceed even under the illumination of 10–50 lux as easily as in the darkness[6]). It has also been reported that the last process of the inductive dark period is also relatively light-stable but this is less stable than the first one[7]). Thus, the critical light intensity which inhibits floral induction varies with the phases of the dark period.

Plants grown in natural daylight receive twilight at the first and the last phase of the dark period, and in both of them the processes inducing flowering are relatively stable to light. Therefore, twilight length must be taken into consideration to define the natural day length for the photoperiodic induction.

In the present investigation, the critical light intensities in the first and the last phases of the dark period for the photoperiodic induction are examined with *Pharbitis* seedlings under natural daylight condition.

Material and Methods

Material used was seedlings of *Pharbitis Nil*, strain " Violet ". Photoperiodic behaviour of the seedling of this plant was reported recently by Kujirai and Imamura[8]).

Seeds were treated with conc. H_2SO_4 for 40–50 minutes, washed in running water for about one day, and spread on moistened sand. Two days after the treatment with H_2SO_4, germinating seeds were selected for uniformity and sown in $30 \times 20 \times 10\ cm^3$. wooden boxes filled with garden soil. In each box 60 plants were placed in 4 rows, and grown under continuous illumination supplemented with incandescent light at night. Two days after the sowing, cotyledons expanded, and one day later the inferior individuals were removed, and the seedlings were subjected to the experimental treatment.

To eliminate the error due to the individuality of the boxes, experiments were designed in such a way that the rows of plants receiving different treatments were growing side by side in one box in randomized order. Light was excluded when desired by covering the plants with light-tight tin boxes.

All experiments were undertaken in a greenhouse, in which the temperature was $25 \pm 2°$ during the treatment. After the treatments plants were kept under continuous

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

illumination supplemented with incandescent light of 500 lux at night. About two weeks later, plants were harvested and dissected under a binocular microscope.

Experiments and Results

Experiment 1. On March 16 and 17, 1959, plants of 4 boxes were treated as follows:

The first lot of the plants consisting of four rows was subjected to natural daylight, but they were covered from 7:00 p. m., when the luminosity was 0 lux, to 5:20 a. m. of the next morning, when the luminosity was also 0 lux. Remaining six lots of the plants, each lot consisting of two rows, were darkened from the times at which luminosities were 500, 200, 100, 50, 10 and 1 lux to the times at which luminosities were 500, 200, 100, 50, 10 and 1 lux, respectively. Thus, the first of the seven lots was subjected to natural daylight and received a dark period of shorter duration than the other six lots. Similar treatments were repeated next day.

From the time at which luminosity was 500 lux to the time at which luminosity was 0 lux, the luminosity of natural daylight was measured by a Mazda lux meter with one minute intervals. The changes in luminosity with time are shown in Fig. 1.

Table 1. Effect of twilight on photoperiodic responses of *Pharbitis* seedlings. Plants were darkened from the varying times of the evening at which luminosities were 0–500 lux to the corresponding times of the next morning. (Treated on March 16 and 17, 1959)

Luminosity of daylight at the start and the end of dark treatment in lux	Duration of dark period		No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
	March 16	March 17				
0	$10^h 20'$	$10^h 20'$	48	97.9	1.9	0
1	$11^h 25'$	$11^h 17'$	25	96.0	2.4	0
10	$11^h 42'$	$11^h 39'$	27	100	2.4	0
50	$12^h 00'$	$11^h 57'$	22	95.4	2.8	0
100	$12^h 11'$	$12^h 08'$	20	100	3.1	5.0
200	$12^h 28'$	$12^h 18'$	21	100	3.8	28.6
500	$13^h 33'$	$13^h 14'$	25	100	4.6	96.0

Results are shown in Table 1. Plants subjected to natural day length initiated 1.9 flower primordia per plant in the average. The plants darkened from the times when the luminosities were 1, 10, 50, 100, 200 and 500 lux to the times having the corresponding luminosities of the next morning initiated 2.4, 2.4, 2.8, 3.1, 3.8 and 4.6 flower primordia per plant, respectively. If the first and the last phase of the inductive dark period proceed under light of 1 lux or less as easily as in complete darkness, flowering response of the plants subjected to natural night length and those darkened from the time at which luminosity was 1 lux to the time having the same luminosity in the next morning have to initiate flower buds to the same extent. But this was not the case.

Thus, it appears that light of only 1 lux suppresses the flowering response to some extent. It had been reported previously[6]), however, that the first process of the inductive dark period can proceed under light of 10–50 lux as easily as in darkness in this plant. It is conceivable that the difference of the photoperiodic responses between the plants subjected to natural day length and those darkened from the time at which luminosity was 1 lux to the time of the same luminosity in the next morning

was due to the effect of morning twilight.

Evidence was already available that the last process of the inductive dark period was relatively light stable but less so than the first one[7]. In the previous experiment, however, light sensitivity of the last phase of the inductive dark period was examined during the 12th to 16th hour of the dark period. The process taking place during the 12th to 16th hour of the dark period is relatively light-stable, but the

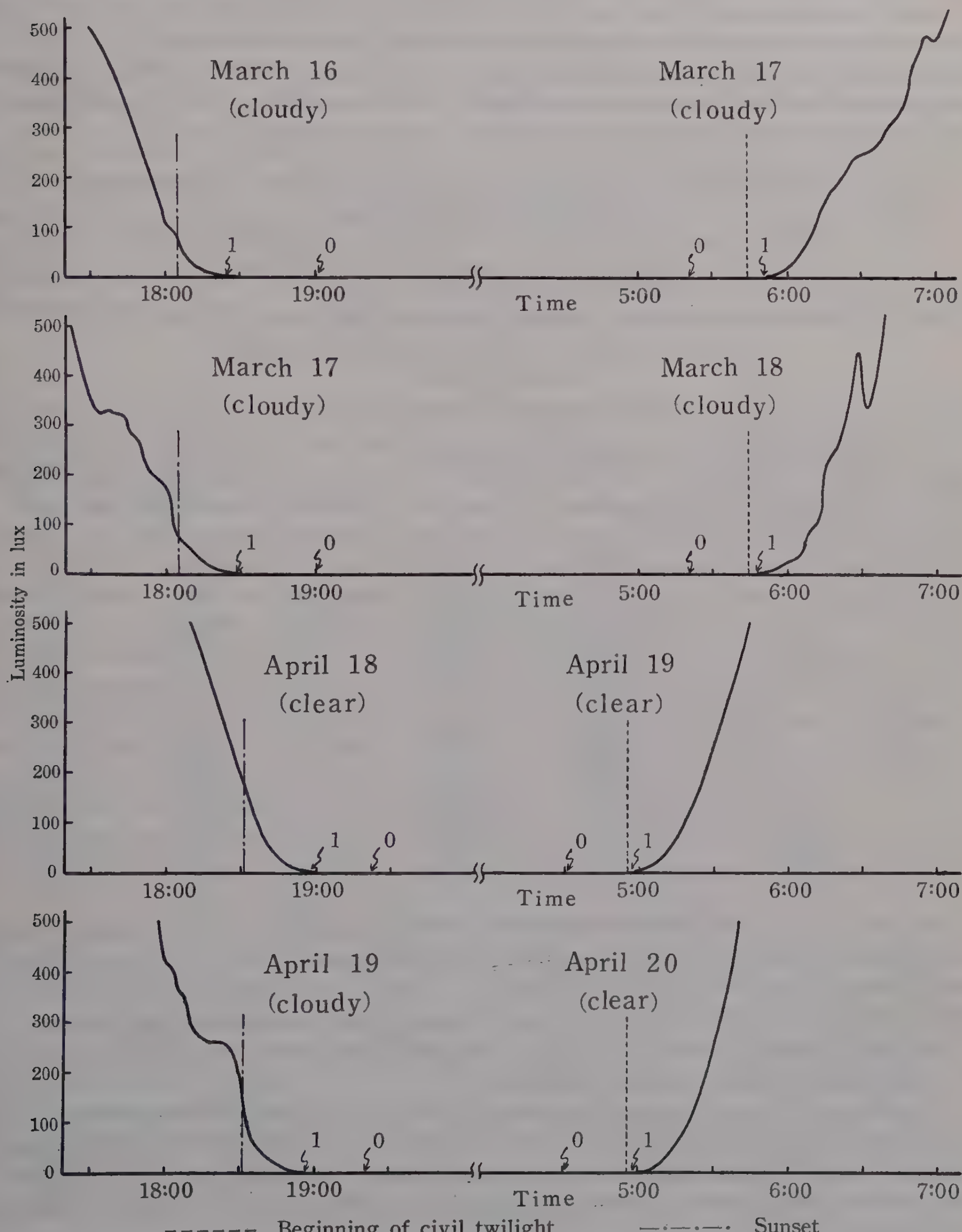


Fig. 1. Luminosity of natural daylight in the evening and in the morning.

process taking place during the 10th to 11th hour of the dark period—this corresponds to the last phase under natural conditions—is considered to be highly light-sensitive. In the next experiment, the effect of twilight in the evening and in the morning was investigated separately.

Experiment 2. On April 18 and 19, 1959, two groups of plants each consisting of 4 boxes were treated as follows:

Group 1: Four rows were subjected to natural daylight but from 7:20 p. m., at the time luminosity was 0 lux, they were covered with light-tight tin boxes. Six lots of plants each consisting of two rows were darkened from the times at which luminosities were 500, 200, 100, 50, 10 and 1 lux in the evening. All light-tight covers were removed at 5:15 a. m. the next morning. Similar treatments were repeated again next day. The luminosities in the evening twilight are shown in Fig. 1.

Table 2. Effect of twilight in the evening and in the morning on photoperiodic responses of *Pharbitis* seedlings.
Group 1: Plants were placed in darkness from the different times of the evening at which luminosities were 0–500 lux until 5:15 a. m. of the next morning.
Group 2: Plants were placed in darkness from 6:30 p. m. until the next morning at various luminosities ranging from 0 to 500 lux.
(Treated on April 18 and 19, 1959)

Group	Luminosity of daylight at the start or at the end of dark treatment	Duration of dark period		No. of plants dissected	% of plants with flower buds	No. of flower buds per plant
		April 18	April 19			
1	0	9h 55′	9h 55′	54	29.6	0.4
	1	10h 19′	10h 19′	29	31.0	0.6
	10	10h 27′	10h 30′	28	17.9	0.2
	50	10h 35′	10h 40′	28	32.2	0.4
	100	10h 40′	10h 43′	25	24.0	0.3
	200	10h 46′	10h 47′	19	44.8	0.6
	500	11h 03′	11h 17′	29	93.1	2.0
2	0	10h 00′	10h 00′	53	5.7	0.1
	1	10h 26′	10h 27′	26	34.6	0.6
	10	10h 35′	10h 37′	27	55.6	0.6
	50	10h 43′	10h 45′	26	42.3	0.5
	100	10h 48′	10h 50′	27	59.3	0.9
	200	10h 55′	10h 55′	29	69.0	1.2
	500	11h 15′	11h 10′	28	100	2.1

As shown in Table 2, plants subjected to natural daylight in the evening, and those darkened from the times at which luminosities were 1, 10, 50, 100 and 200 lux initiated flower primordia to the same extent. Plants darkened from the time at which luminosity was 500 lux initiated significantly more flower buds than others.

Similar experiments which are not represented here, were undertaken on March 29 and 30, 1959, and gave similar results.

These results show that the first phase of the dark process inducing flower primordia can proceed under natural daylight of 1–200 lux as readily as under darkness. Sunset on April 18 occurred at 6:31 p. m., at the time luminosity was about 200 lux. The inductive dark process is considered, therefore, to proceed from the time of sunset or thereabout.

Group 2: All the plants were covered at 6:30 p. m. Four rows were returned

to natural daylight at 4:30 a.m. the next morning, at the time luminosity was 0 lux. Another 6 lots each consisting of 2 rows were subjected to natural daylight from the time at which luminosities reached 1, 10, 50, 100, 200 and 500 lux in the morning. Luminosities in the morning twilight are shown in Fig. 1.

As shown in Table 2, with delayed removal from darkness, the number of flower primordia initiated was increased. The plants subjected to natural daylight in the morning from the time at which luminosity was 0 lux initiated only 0.1 flower bud per plant, but those kept in the dark until the time at which luminosity was 1 lux initiated 0.6 flower bud per plant. Thus, only 1 lux of natural daylight inhibits the last phase of the dark process. Similar experiments were undertaken on March 29 and 30, 1959, and gave similar results. It appears that the last phase of 10- to 11-hour dark period is very sensitive to natural daylight, and that the difference of the flowering responses between the plants subjected to natural day length and those placed in the dark from the time at which luminosity was 1 lux to the corresponding time of the next morning in Experiment 1, is attributable to the effect of morning twilight.

Luminosity at the beginning of civil twilight is about 1 lux. Therefore, it is considered that the inductive dark period ends before the beginning of civil twilight.

Discussion

From the present experiments, it appears that the inductive dark period in *Pharbitis* plants begins when the luminosity is about 100–200 lux—this corresponds to the time of sunset—and ends at or before the beginning of civil twilight.

Some preliminary experiments which are not presented here showed that the first two hours of 12-hour dark period can proceed to some extent even under the light of 200–1000 lux, and that the last two hours of 12-hour dark period can also proceed to some extent under the illumination of 0.5–10 lux. In these cases the flowering responses are decreased with increasing light intensities.

Plants cultured under natural daylight are subjected to light whose intensity decreases gradually in the evening, and increases gradually in the morning. The inductive dark process is also assumed to begin and end gradually under these conditions. Therefore, the beginning or the end of the inductive dark period can not be determined clearly. But generally it may be said that the inductive dark period begins at the time of sunset and ends at the beginning of civil twilight.

The luminosity at the sunset or the beginning of civil twilight varies considerably with the weather. Some data obtained at Kyoto are presented in Table 3. In

Table 3. Effect of weather on biological day length.

Date (1959)	March 16	March 17	March 30	March 31	April 1	April 18	April 19
Weather	cloudy	cloudy	cloudy	rain	clear	clear	clear
① Astronomical day length	11h 58′	12h 00′	12h 29′	12h 30′	12h 33′	13h 09′	13h 10′
② Length from the beginning of civil twilight to sunset	12h 23′	12h 25′	12h 53′	12h 55′	12h 58′	13h 34′	13h 36′
③ Biological day length for flowering of *Pharbitis*	12h 04′	12h 10′	12h 17′	12h 20′	13h 05′	13h 33′	13h 31′
④ ③—①	+ 6′	+10′	−11′	−10′	+32′	+24′	+21′
⑤ ③—②	−19′	−15′	−36′	−35′	+ 7′	− 1′	− 5′

this table, biological day length for flowering of this plant was regarded as the period from the time at which luminosity was 1 lux in the morning to the time at which luminosity was 200 lux in the evening. On clear days the biological day length is longer than the astronomical day length by some 20–30 minutes, and it is approximately equal to the length of time from the beginning of civil twilight to sunset. On cloudy days, biological day length is nearly equal to astronomical day length.

The time at which luminosity is 200 lux in the evening is earlier than sunset by some 30 minutes on cloudy days, but the difference between the time at which luminosity is 1 lux and the beginning of civil twilight varies with the weather only a little and rarely exceeds 15 minutes. Thus, the weather affects mainly the end of biological day length.

From these points of view, the biological day length for *Pharbitis* plants at the summer solstice is about 14.5–15 hours at Kyoto. The critical day length of *Pharbitis Nil* is also about 15 hours[5,8]. *Pharbitis* plants cultured in the field initiate flower primordia late in June or early in July, at which time the day length is longest. Before June, the day length is shorter than the critical day length of *Pharbitis* plants. Why do *Pharbitis* plants cultured in the field under natural conditions not initiate flower primordia until the end of June or beginning of July? It appears that the night temperature is too low before June. Optimum night temperature for flower initiation of *Pharbitis* plants is about 25°, and the photoperiodic response is reduced with lowered temperature at night.

Pharbitis plants subjected to fully inductive short photoperiod initiate a terminal flower bud and stop further growth. But plants cultured under natural day length are subjected to nearly critical photoperiod every day in June and July, and under these conditions flower buds are initiated successively in the leaf-axils, and no terminal flower bud is formed. Thus, under natural conditions, *Pharbitis* plants grow vigorously and initiate lateral flower buds.

Whether the results obtained here can or can not be applied to other plants is uncertain, and remains to be examined.

Summary

Effect of natural twilight on photoperiodic responses of *Pharbitis* seedlings was examined.

1) The inductive dark process proceeds from the time at which luminosity is about 100–200 lux.

2) The inductive dark process is inhibited by morning twilight of 0–1 lux.

Generally, it may be said that the biological day length for flowering of this plant begins at the beginning of civil twilight in the morning and ends at the time of astronomical sunset.

On cloudy days, however, the biological day length becomes shorter than that on clear days by some 30 minutes and becomes nearly equal to the astronomical day length.

Grateful acknowledgment is given to Prof. S. Imamura for his suggestions and criticisms.

References

1) Borthwick, H. A., and Parker, M. W., Bot. Gaz. **100**: 374 (1938). 2) Sasamura, S., Bull. of

the College of Agr. Utsunomiya Univ. **3**: 334 (1952). 3) Withrow, R. B., and Benedict, H. M., Plant Physiol. **11**: 225 (1936). 4) Imamura, S., and Takimoto, A., Bot. Mag. Tokyo **68**: 235 (1955). 5) Takimoto, A., and Ikeda, K., ibid. **72**: 137 (1959). 6) ——, and ——, ibid. **72**: 388 (1959). 7) ——, and ——, ibid. **73**: 91 (1960). 8) Kujirai, C., and Imamura, S., ibid. **71**: 408 (1958).

摘　　要

アサガオの花芽形成を支配する光条件について
VI. 自然薄明の影響

滝本　敦・池田勝彦

自然条件下で，アサガオ子葉の日長感応に有効な日の長さを決める目的で実験を行なった.

すでに発表したように[6]，暗期反応の初期段階は比較的光に安定で，夕方 100～200 ルックスになった時（大体天文日没時）より暗期反応がはじまるものと考えられる．これに対して，暗期反応の後期段階（暗期開始後 10～11 時間目）は非常に光に敏感で，1 ルックス以下の光でかなり抑制され，大体朝 1 ルックスになった時（大体常用薄明起時）に暗期反応が終了するものと考えてよさそうである.

すなわち日長反応に有効な日長は天文日長より約 30 分長い．しかし曇天の日には天文日長と，日長反応に有効な日長はほぼ等しくなる．（京都大学農学部応用植物学研究室）

Nuclear and Cell Divisions in Zoospore Formation of *Ulva pertusa* Kjellman

by Hiroshi YABU* and Jun TOKIDA*

Received October 9, 1959

Cytological studies of the green algae belonging to the family Ulvaceae have been reported to date by four investigators, viz., Carter[1]), Føyn[2,3]), Ramanathan[4]), and Niizeki[5]). Meiosis in those algae has been established to take place in zoospore formation by Føyn[3]) and Ramanathan[4]).

Among the Japanese species of this family, *Enteromorpha Linza* (L.) J. Ag. is the only one that has hitherto been treated cytologically[5]). In the present paper, the writers wish to report some results of their recent investigation on the nuclear and cell divisions in zoospore formation of *Ulva pertusa* Kjellm.

The material was collected in the middle of April, 1955 at Nanaehama near Hakodate. It was brought to the laboratory and kept alive for some time in glass vessels containing sea-water. The fixation of the material was done from 8 p. m. of the collection day to 6 a. m. of the following day at intervals of 30 minutes, with two kinds of fixing fluids, viz., Navashin's solution and Flemming's weaker solution made up, of course, with sea-water. Three to four hours were sufficient for fixing. Sections were cut 2–3 μ in thickness by the paraffin method at right angles or parallel to the surface of the thallus, and were stained with Heidenhain's iron haematoxylin. In a section cut parallel to the thallus surface, the nuclei were likely to be confused with the pyrenoids, so the observation here described was made with the sections cut at right angles to the thallus surface.

Thallus cells adjacent to the fertile area of the zoospore-producing material always contained a single nucleus near the center of the cell cavity and a single nucleolus within each nucleus. In the beginning of the nuclear division, the nucleus and the nucleolus gradually increased in size. Thin chromatin threads soon appeared in the nuclear cavity and the synapsis stage set in. Then the threads spread to fill up the whole cavity (Fig. 5). In diakinesis stage, a few V-shaped chromosomes were observed (Fig. 6). Thirteen bivalent chromosomes were counted in this and the following stages (Figs. 6–8). This haploid chromosome number coincides with that counted by Føyn[3]) in *Ulva lactuca*, but not with those reported by Carter[1]) in *Ulva lactuca* and by Ramanathan[4]) in *Enteromorpha compressa* var. *ligulata*. It also differs from the chromosome number reported recently by Niizeki[5]) in his materials of *Enteromorpha Linza* collected in Tokyo Bay. He reports that his materials were considered to be a strain represented by haploid generation only, and that they produced biflagellate asexual swarmers without meiosis and 12 chromosomes were counted at metaphase of the first nuclear division in the swarmer formation.

The nucleolus and the nuclear membrane disappeared completely in metaphase and reappeared in telophase (Fig. 11). In side view of the metaphase, a small centrosome-like body was clearly observed at each pole of the spindle (Figs. 9 and 22). Equatorial plate of the first nuclear division was always parallel to the thallus surface, therefore the cell division following the first nuclear division was likewise parallel to the thallus surface. In the daughter cells thus formed, the second nuclear division started

* Phycological Laboratory, Faculty of Fisheries, Hokkaido University, Hakodate, Japan.

Figs. 1–20. Nuclear and cell divisions in fertile cells of the sporophyte of *Ulva pertusa*: 1–4, synapsis; 5, spireme; 6, diakinesis; 7–8, late prophase; 9, metaphase; 10, anaphase; 11, interkinesis; 12–15, the second nuclear division; 12, late prophase; 13, metaphase; 14, three-cell stage; 15, four-cell stage; 16–17, metaphase of the third nuclear division; 18, eight-cell stage; 19, sixteen-cell stage; 20, zoosporangium after the liberation of zoospores, three of which still remain within. (Figs. 1–20, ×1870)

immediately (Figs. 12 and 13). The second cell division was either at a right angle or parallel to the thallus surface (Figs. 14–17). The second and the succeeding nuclear divisions in all of the cells produced in a young zoosporangium generally took place simultaneously (Figs. 12, 13, 16 and 17), but sometimes with a slight discrepancy as shown in Fig. 14. As a result of the successive third and fourth nuclear and cell divisions, 16 small cells were eventually formed within a zoosporangium, and each of those small cells was converted into a quadriflagellate zoospore (Fig. 20). As for the number of the zoospores formed in each sporangium, Printz[6]) gives "4–8" in his

Figs. 21–25 and 27. Photomicrographs of microtome sections through fertile parts of the sporophyte of *Ulva pertusa*: 21, part of a section showing cells at the stages of the first and second nuclear divisions; 22, a single cell showing side view of the metaphase of the first nuclear division; 23, part of a section showing cells mostly at the stages of the second and third nuclear divisions; 24, part of a section showing mature zoosporangia; 25, part of a section stained with aniline blue showing mature zoosporangia containing 16 zoospores; 27, part of Fig. 25, enlarged. (Figs. 21, 23 and 24, ×680; Fig. 22, ×1400; Fig. 25, ×425)

Figs. 26 and 28. Photomicrograph of a microtome section, stained with aniline blue, through a fertile part of a gametophyte of *Ulva pertusa*, showing mature gametangia containing 32 gametes; 28, part of the preceding figure, enlarged. (Fig. 26, ×425; Fig. 28, ×850).

general account of the family Ulvaceae. However, the writers have ascertained that *Ulva pertusa* collected at Nanaehama produced 16 zoospores in each sporangium (Figs. 19 and 25). By the way, the number of gametes produced in each gametangium of this species was 32 in the writer's specimens from Nanaehama (Fig. 26), while it was described by Printz[6] in Ulvaceae as "8 (seltener 4 oder 16)."

Summary

The zoosporophyte of *Ulva pertusa* Kjellm. collected near Hakodate is reported here to have been proved cytologically to undergo meiosis in zoospore formation as previous authors have already reported in other species of *Ulva* and *Enteromorpha*. Haploid chromosome number was established to be 13. A centrosome-like body was

present at each pole of the spindle in the metaphase of meiosis. Each sporangium produced 16 quadriflagellate zoospores.

The present study was supported in part by a grant in aid for Miscellaneous Scientific Research from the Ministry of Education.

References

1) Carter, N., Ann. Bot. **40**: 665 (1926). 2) Føyn, N., Ber. Deutsch. Bot. Ges. **47**: 495 (1929). 3) ——, Arch. f. Protistenk. **83**: 154 (1934). 4) Ramanathan, K. R., Ann. Bot. N. S. **3**: 375 (1939). 5) Niizeki, S., Nat. Sci. Rept. Ochanomizu Univ. **8**: 45 (1957). 6) Printz, H., "Chlorophyceae" in A. Engler, Natürl. Pflanzenfam. 2. Aufl. 3 Bd. 463 pp. Leipzig (1927).

摘　　要

アナアオサの游走子形成の際の核および細胞分裂

籔　　凞・時 田　郇

函館付近で採集したアナアオサの胞子体は細胞学的にしらべた結果，アオサ属とアオノリ属の外国種ですでに報告されているところと一致して，游走子形成の際に減数分裂を行なうことをたしかめた．染色体数は $n=13$ であった．核分裂中期の側面観で紡錘体の両極に中心体が明らかに見られた．核および細胞の分裂は4回つづけて行なわれ，游走子嚢内には結局 16 個の游走子が形成される．游走子は鞭毛4本を有する．比較のため配偶体もしらべたところ配偶子嚢内に鞭毛2本の配偶子 32 個を形成することをたしかめた．緑藻アオサ科の日本産の種類で減数分裂を観察した報告は今日まで他に知られていない．(北海道大学水産学部水産植物学教室)

The Occurrence of Gibberellin-like Substances in Cereal Grasses

by Yutaka MURAKAMI*

Received October 13, 1959

Unlike the oat coleoptile, which reacts sensitively to light, the green rice leaf does not bend toward the source of the light. In the previous paper[1]), it was demonstrated that the sucrose-induced growth in the length of leaf sheath sections isolated from the basal region of the rice leaf was inhibited by auxin but stimulated by gibberellin A. Moreover, the author[2]) found that the ether extract of immature bean seeds contains a growth substance which promotes the growth of the intact foliage leaf of rice plants. These facts led the author to suspect that a growth factor or factors other than auxin may be operating in the growth regulating system of rice plants. On the other hand, the gibberellins, which were initially isolated from the metabolic product of the fungus *Gibberella fujikuroi*, have now been established to occur in higher plants by several researchers[3,4,5]) including the author[6]). Some of the active substances have been identified as gibberellin A_1[7,8,9]). Hence, it seemed interesting to examine whether or not the growth factor such as gibberellin A is detected in rice plants.

This paper aims to present the direct evidence for the occurrence of gibberellin A or its similar active substances in cereal grasses.

Materials and Methods

The occurrence of gibberellin-like substances was examined on shoots, roots, and immature grains of rice, wheat, and maize.

Rice (var. Aichi-asahi) was grown in the paddy field in order to take samples of shoots and immature grains. 230 shoots (total fresh weight 184 g.) at the fifth leaf stage, and 11,130 grains (total fresh weight 260 g.) at the milk ripe stage were harvested for the extraction. 620 g. fresh weight of roots were detached from the plants at the tenth leaf stage, which were grown in water culture.

Wheat (var. Akasabishirazu) was grown in the field. 220 shoots (total fresh weight 220 g.) at the seventh leaf stage and 2,400 grains (total fresh weight 95 g.) at the similar ripe stage to that of the rice plant were harvested for the extraction.

Maize (var. Chôkô 161) was also grown in the field. 190 shoots (total fresh weight 220 g.) and 150 g. of roots were harvested at the fourth leaf stage. Roots were washed free from soil. 1,620 grains (total fresh weight 290 g.) were harvested at the milk ripe stage.

The methods of plant extraction, chromatography, and bioassay are similar to those used in the previous papers[6,10]).

Extraction: Each plant material, immediately after harvesting was ground in a blendor with eight times its fresh weight of 70% acetone and this blended mixture was allowed to stand overnight at room temperature. The homogenate was then filtered off the solid material with suction, and the residue was extracted once more in a similar manner. The combined filtrates were evaporated under reduced pressure. The resulting aqueous residue was adjusted to pH 7.0 with sodium hydroxide and filtered. The filtrate was acidified to pH 2.0 with phosphoric acid and extracted with three

* National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo, Japan.

portions of ethyl acetate. The ethyl acetate solution was then extracted with three portions of 1 M phosphate buffer at pH 7.0. This phosphate buffer solution was acidified to pH 2.0 with phosphoric acid and extracted again with three portions of ethyl acetate. The ethyl acetate extract was dried by anhydrous sodium sulfate overnight and then the solvent was distilled off under reduced pressure. The resulting residue was taken up in a small volume of acetone and was subjected to paper chromatography.

Paper chromatography and bioassay: Ascending paper chromatography was carried out on Tôyô No. 50 filter paper at about 28° with the mixture of *iso*-propanol /water/ammonia (10:1:1) until the solvent front was 32 cm. from the starting line.

The developed chromatogram was dried and divided transversely into 16 equal strips. Each strip was again cut into fine segments, placed in beakers 2 cm. in diameter and 7 cm. in height containing 1.5 ml. of water, and bioassayed by the author's rice seedling method, which is specific to gibberellin A. Briefly, five rice seedlings (var. Aichi-asahi), whose coleoptiles attained about 1mm., were planted on each paper piece and allowed to grow under ordinary daylight conditions at about 30°. They were supplied with 0.5 ml. water every day. The length of the second leaf sheath was measured after 7 days. A result typical of the rice seedling method is given in Fig. 1.

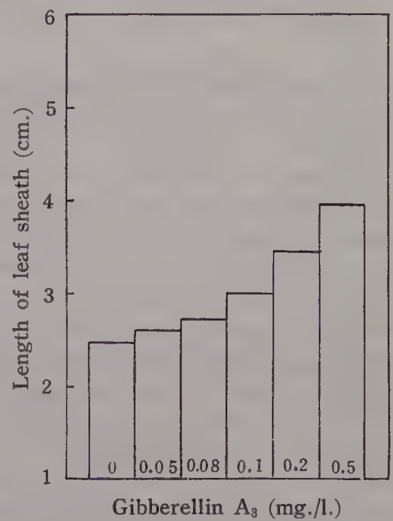


Fig. 1. Response of the second leaf sheath of rice seedling to gibberellin A_3.

Results

The occurrence of gibberellin-like activity in cereal grasses was examined in the extracts obtained from shoots, roots, and immature grains of rice, wheat and maize. The results are summarized in the form of histograms showing the length of leaf sheath of the test rice seedling (Fig. 2). It will be seen from these histograms that the gibberellin-like activity is detected in all tissues of cereal grasses examined.

On the extracts from rice shoots (Fig. 2-A), roots (Fig. 2-B), and grains (Fig. 2-C), and wheat grains (Fig. 2-E), the gibberellin-like activity was spread over the lower half of each chromatogram with the maximum peak near R_f 0.3. When gibberellin A alone is developed with ammoniacal *iso*-propanol and bioassayed with rice seedlings, its growth-promoting activity is found near R_f 0.7[2,6]). Since impurities in plant extracts cause variations in the R_f value of gibberellin A, further investigations are required to confirm whether the chemically known gibberellin A is responsible for these growth-promoting activities.

On the histograms of the extracts obtained from wheat shoots (Fig. 2-D), and maize shoots (Fig. 2-F), roots (Fig. 2-G) and grains (Fig. 2-H), another zone of growth-promoting activity was detected at the solvent front in addition to that already mentioned. Very recently, Simpson[11]) has found a similar result with etiolated wheat seedlings. He has reported that the extract obtained from etiolated wheat seedlings, after chromatography on paper with ammoniacal *iso*-propanol and bioassay with a dwarf pea, gives two zones of growth promotion and that one of them is located at the solvent front.

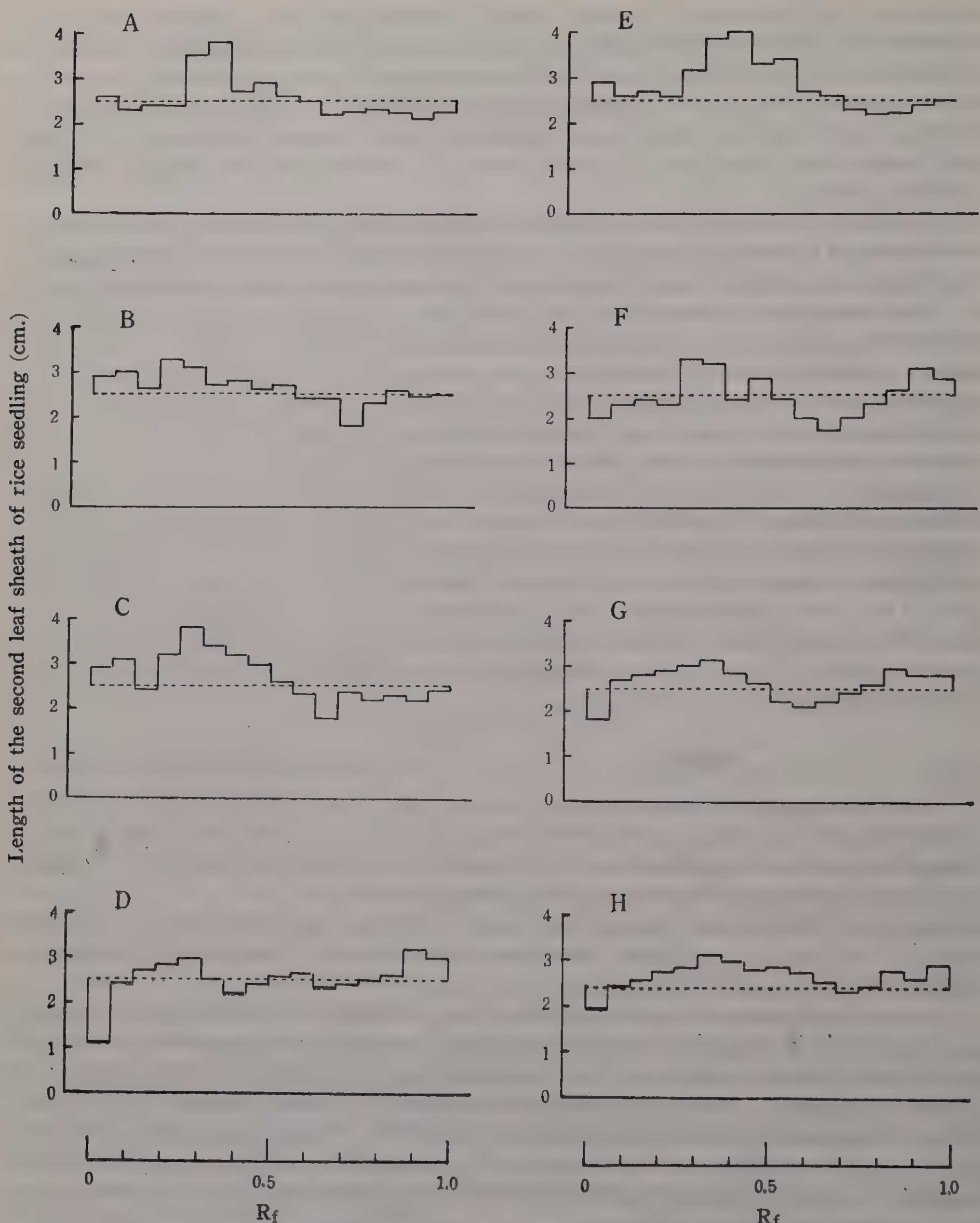


Fig. 2. Histograms showing gibberellin-like activity of extracts of cereal grasses after paper chromatographic development with ammoniacal isopropanol. Broken lines denote water controls. A, rice shoots; B, rice roots; C, immature rice grains; D, wheat shoots; E, immature wheat grains; F, maize shoots; G, maize roots; H, immature maize grains.

The approximate amounts of substances with gibberellin-like activity were estimated by comparison with a standard gibberellin A_3 bioassay. The total active substances in 100 g. fresh weight of rice, wheat and maize grains were approximately equivalent to 0.7, 1.5, and 0.5 μg. gibberellin A_3, respectively. In their shoots or

roots, there was a much lower concentration of gibberellin-like substances. The concentration was roughly estimated to be 0.1–0.5 μg. per 100 g. fresh weight. Radley[5]) has found the similar amounts of gibberellin-like substances in pea shoots, while McComb and Carr[12]) have found 2.1 μg. gibberellin A_3 equivalents per 100 g. fresh weight of pea shoots. It should be noted that these results are only semi-quantitative.

Discussion

The present experimental data indicate that the gibberellins, which are characterized by their capacity to induce the elongation of intact green plants, are widely distributed in various tissues of cereal grasses and operating in their growth regulating system.

Numerous height varieties of many cultivated plants are in existence and their inheritable size is generally believed to be related to the metabolism of auxin such as indoleacetic acid. Indeed, auxin, which was driven from the study of a curvature of the coleoptile, has been shown to promote markedly the growth of the section of coleoptiles. Thus the oat or wheat coleoptile section is most widely used for the survey of naturally occurring growth substances in plants. There are, however, little records of auxin applications increasing growth of intact green plants. It is generally assumed that this absence of growth-promoting activity would be expected if auxin levels in green plants are not suboptimal, but there is no direct evidence for the supra-optimal presence of free auxin in them. On the contrary, no free auxin, which is diffusible into agar block, could be detected in the green leaf of rice plants[1]). Further, supplying an auxin, 2, 4-dichlorophenoxyacetic acid, to dwarf green rice plants may initiate growth responses such as epinasty but, depending on the amount supplied, may check normal growth as would a toxin.

Gibberellin A promotes the growth of intact green plants and, morever, there is now a considerable body of evidence on the occurrence of gibberellin A or its similar active substances in a wide range of plant tissues. These facts suggest that the inheritable height of plants is not related to a hormone of auxin but that of gibberellin. Therefore, the bioassay of the hormone controlling growth should be carried out by the use of the intact green plant.

Also, it has been known that gibberellin A has a slight activity in the oat or wheat coleoptile section test[13,14]). The possibility must be recognized, therefore, that the existence of gibberellin-like substances in plant extracts may occasionally be demonstrated under the name of auxin, if the oat or wheat coleoptile section test is used for the bioassay of native growth hormones.

Summary

The occurrence of gibberellin-like substances in extracts of shoots, roots and immature grains of rice and maize, and shoots and immature grains of wheat was examined by the rice seedling method after chromatography on paper with ammoniacal *iso*-propanol.

Gibberellin-like substances were present in amounts approximately equivalent to 0.1–0.5 μg. of gibberellin A_3 per 100 g. fresh weight in shoots and roots, and 0.5–1.5 μg. in immature grains.

The necessity for the use of intact green plants for bioassay of hormones, which are responsible for elongation in shoots, is discussed.

References

1) Murakami, Y., Bot. Mag. Tokyo **69**: 258 (1956). 2) ——, ibid. **70**: 376 (1957). 3) Phinney, B. O., West, C. A., Ritzel, M., and Neely, P. M., Proc. Nat. Acad. Sci. **43**: 398 (1957). 4) Lona, F., L'Atneo Parmense **28**: 111 (1957). 5) Radley, M., Ann. Bot. **22**: 297 (1958). 6) Murakami, Y., Bot. Mag. Tokyo **72**: 36 (1959). 7) MacMillan, J., and Suter, P. J., Naturwissenschaften **45**: 46 (1958). 8) Kawarada, A., and Sumiki, Y., Bull. Agr. Chem. Soc. Japan **23**: 343 (1959). 9) West, C. A., and Phinney, B. O., J. Am. Chem. Soc. **81**: 2424 (1959). 10) Murakami, Y., Bot. Mag. Tokyo **72**: 438 (1959). 11) Simpson, G. M., Nature **182**: 528 (1958). 12) McComb, A. J., and Carr, D. J., Nature **181**: 1548 (1958). 13) Hayashi, T., and Murakami, Y., J. Agr. Chem. Soc. Japan **27**: 797 (1953). 14) Brian, P. W., Hemming. H. G., and Radley, M., Physiol. Plantarum **8**: 899 (1955).

摘　　要

穀類に含まれるジベレリン類似物質

村　上　　浩

イネ，小麦，トウモロコシの茎葉，根，未熟種子よりえた抽出物について，ジベレリンに作用の類似している物質の分布を，ペーパークロマトグラフィーにイネ苗試験法を併用して調査した．茎葉と根については，生重量 100 g. 当り，約 0.1–0.5 μg.，未熟種子では約 0.5–1.5 μg. のジベレリン A_3 に相当する作用物質を検出しえた．なお，伸長を支配するホルモンの検出には，イネ苗試験法のように，“intact” な植物体を試験植物とする必要性を強調した．(農林省農業技術研究所)

ベニシダ配偶体の形態分化と生長物質*

堀　田　康　雄**

Yasuo HOTTA**: Morphological Differentiation and Growth Substance in the Gametophyte of *Dryopteris erythrosora**.

1959 年 3 月 23 日受付

Indole acetic acid (IAA) は高等植物の形態分化に関与する因子の一つとされている．シダ類の配偶体の後期の形態形成においても，IAA は重要な働きをしていることが Albaum[1,2]) によって見出されている．

筆者らはさきに，ベニシダを用いてN化合物と配偶体の形態分化との関係[3])，および，蛋白・リボ核酸 (RNA) と分化との関係[4,5,6]) を報告した．本報においては，ベニシダ配偶体の形態分化，とくに二次元分化（線状の原糸体から平面状の葉状体への転換）に伴なう体内の生長物質の変化を，IAA を中心としてしらべた．さらに，培養液に生長物質を加えて配偶体の形態分化・生長におよぼす影響をしらべた．

材料と方法

ベニシダ配偶体を材料として用いた．形態分化の諸過程および培養法は前報[3]) と全く同じである．

生長物質の測定法

1)　Ether extractable auxin の定量．約 10 倍量の過酸化物を除いた ether を用い，暗所 (5°) で 20 時間抽出する．約 3500 *g* 5 分間遠心し約 2 倍量の ether で洗い上澄を合せる．これに重曹で pH 8.6 にした水を等量加え，glucose で飽和して充分に振り，ether 分画を “アルカリ分画” とする．水の分画を酒石酸で pH 7.0 とし，等量の ether を加えて振り，ether 分画を “中性分画” とする．水の分画を酒石酸で pH 3.0～3.5 とし，等量の ether を加えて振り，のち，ether 分画をとる．この操作を二度繰返したのち ether 分画を合せて “酸性分画” とする．各分画は，Ether を除いて少量の水にとかし，Went の *Avena* coleoptile の curvature test と straight growth test で生長物質の定量を行なった．両テストの結果得られた値はおよそ一致していた．

2)　Bound auxin の定量．Ether extractable auxin を抽出した残渣に約 10 倍量の 0.1 N NaOH を加え，70° 30 分間加熱する．分解物を冷却後沪過し，沪液を 2 N HCl で pH 8.6～9.0 とし，glucose 飽和にし，等量の ether を加えてよく振り，ether 分画を “アルカリ分画” とする．“中性分画”・“酸性分画” は 1) と同様にして得た．生長物質の定量も 1) と同様にした．

3)　生長物質の分析．各分画の ether を除いたものに 0.15 ml の蒸溜水を加え，0.05 ml. の溶液を sample としてペーパークロマトグラムにした．方法は一次元上昇法，沪紙は東洋沪紙 No. 51．展開剤は 70％ ethanol と isopropanol–ammonia–water (10：1：1) を用いた．展開したクロマトグラムは 10 部に分け，蒸溜水で生長物質を流し出し，*Avena*-test にかけた．クロマトグラムの一部は呈色反応に用いられた．発色はSalkowski 試薬と p–amino–benzaldehyde–HCl 法によった．

結果および考察

1)　Diffusible auxin.

高等植物には細胞間や組織間を移動する生長物質が存在し，それが形態分化に関係をもっていることは一般に認められている．したがって，ベニシダ配偶体にもそのような生長物質の存在が予想される．

第 1 に，配偶体から体外に出される生長物質の有無をみた．5 細胞期，二次元分化後 10 日および 100 日目のもの 100 mg. を小寒天片 (2 mm. × 2mm. × 4 mm.) にのせ，15 時間放置し，*Avena*-test にかけた．生長物質は検出されなかった．

---

*　本報の一部は，日本植物学会中部支部例会 (1957) において報告した．

**　名古屋大学理学部生物学教室　Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan.

第2に，細胞内を移動する生長物質の存在をみるために，上と同じ時期の配偶体をいくつかの部分に切ったものを用い，同様に寒天片にのせ 1, 15, 24 時間後に *Avena*-test を行なった．

さらに，IAA-oxidase の作用を阻止するため Steeves *et al.*[7]) の方法にしたがって実験を繰返した．生長物質の存在は認められなかった．

この結果は，Albaum の結果と異なる．この原因としては，シダの種類の差よりも彼はほとんど完熟した配偶体を用いていることにあると考えられる．とにかく，ベニシダにおいては，「Diffusible auxin は配偶体形成の途中には全く存在しない」と結論される．

2) Ether extractable auxin と bound auxin の量的変化．

Ether extractable auxin は高等植物に広く存在していることが知られており，Steeves *et al.*[7]) によってシダ胞子体にも存在することが確められている．bound auxin については今まで余り知られておらず，その存在場所，作用機作など，何もわかっていないといえる．最近，生長物質は蛋白質と結合した形で作用することが知られた[8,9])．

Ether extractable auxin と bound auxin の量的変化は Fig. 1 に示した．この二種の生長物質は

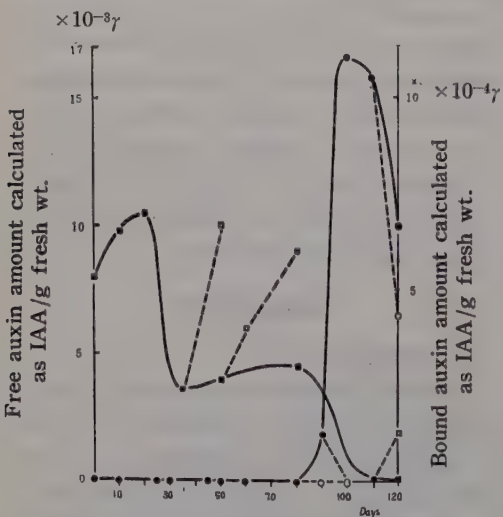


Fig. 1. Quantitative changes of ether extractable auxin (●——●, ○---○) and bound auxin (■——■, □---□).
——: Normal growth.
---: Artificial one-dimensional growth.

“酸性分画” だけに出て来て，他の分画には全く抽出されない．

Ether extractable auxin は胞子，一次元生長，および二次元生長の前半には全くみられず，二次元生長の後半に出現し，100 日目で最大量である．Ether extractable auxin は二次元分化とは直接結びついていないことを示し，むしろ分裂域の活動の高まりと直接的な関係をもっていることを示す．

Ether extractable auxin の変動の様子は，alcohol extractable fraction のアミノ酸分析の結果[3]) で示された tryptophane の変動と大体同じであることを指摘しておく．

Bound auxin の方は，胞子にも可成りの量存在し，発芽後一次元生長中に増加し，二次元生長に移行すると一旦急激に減少するが再び徐々に増加し，二次元生長後半に分裂域の活動が高まると再び急激に減少する．Bound auxin は恐らく形態分化，とくに二次元分化と密接な因果関係をもっているものと思われる．

つぎに，分化との関係をより詳しく知るために人為的一次元生長を起させ，これを利用した．すなわち，一旦二次元分化したものを培地中からN源を完全に除くか，光を弱くして再び人為的一次元生長に戻したものを用いた．この場合，ether extractable auxin は減少し消失するが，bound auxin は再び増加して高い濃度となる．

「一次元生長は細胞内の bound auxin の高い濃度によって，二次元生長は比較的低い濃度によって特徴づけられる．Ether extractable auxin は分裂域の活動の程度とは関係があるが，二次元分化とは無関係のようである」と結論される．

3) Bound auxin の質的変動（ペーパークロマトグラフィーによる分析）．

Bound auxin が二次元分化と因果関係があるらしいことが解ったので，その質的変化をしらべてみた．3細胞期，5細胞期，二次元分化後 10 日目および 60 日目の 4 時期について，bound auxin のペーパークロマトグラフィーによる分析を行なった (Fig. 2, Fig. 3)．一次元生長については，3細胞期と5細胞期の場合は，ほぼ同じ結果であったので，5細胞期の場合のクロマトグラムを Fig. 2 に示し，これを一次元生長期のパターンとした．二次元生長については，二次元分化後 10 日

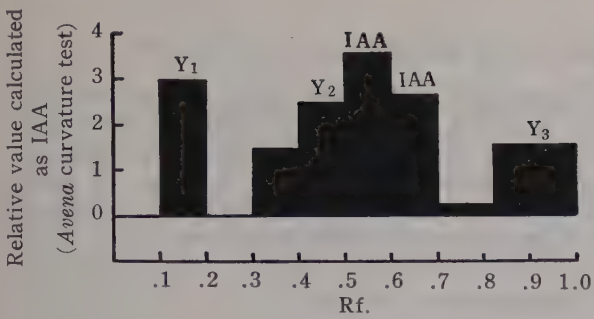


Fig. 2. Paper-chromatographic pattern of the bound auxin in one-dimensional and artificial one-dimensional growth. (Eluent: 70% ethanol).

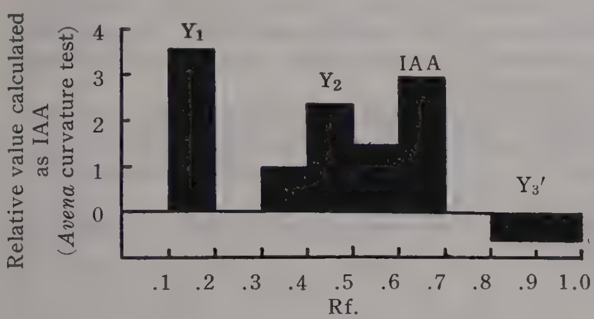


Fig. 3. Paper-chromatographic pattern of the bound auxin in two-dimensional growth. (Eluent: 70% ethanol).

目と 60 日目もおよそ同じ結果を得たので，10 日目の場合のクロマトグラムを Fig. 3 に示し，これを二次元生長期のパターンとした．

一次元生長期には IAA と Y_1, Y_2, Y_3 という 3 つの生長促進物質が見出された．二次元生長では IAA と生長促進物質 Y_1, Y_2 と生長抑制物質 Y_3' の存在が知られた．二次元生長で bound auxin の量的減少がみられたのは Y_3 の消失と Y_3' の出現に起因することが大である．

Y_1, Y_2, Y_3, Y_3' が何であるか，今のところ判らない．ただし Y_3 と Y_3' は非常によく似た物質である可能性が高い．分子の極く一部の変化によって生長促進作用を示したり，抑制作用を示したりするものらしい．もしこれが正しいとすると，「二次元分化のさいには，生長物質の質的差はないか，またあつても極めて部分的なものである」と思われる．

4) 培地に生長物質を加えた場合の形態分化におよぼす影響．

規準培養液，すなわち 1/5 にうすめた Knop 液[3]に IAA を加えた場合と，phenyl acetic acid (PAA) を加えた場合，配偶体の形態分化にどのような影響がみられるかをしらべた．二次元分化については IAA では規準培養と差がほとんどみられない．PAA では早く二次元分化が始まる（一次元生長期の細胞数は規準培養液と差がない）．IAA, PAA ともに高濃度では分枝その他の異常がみられる．

5) ベニシダ配偶体の二次元分化と体内 auxin の変動との関係を一括して考察してみよう．

(i) Diffusible auxin は配偶体の初期には検出されないから，まず問題とならない．(ii) Ether extractable auxin は分裂域から生産されて，配偶体後期の分化，生長に何らかの役をしていると思われるが，二次元分化自体とは無関係である．

(iii) Bound auxin は二次元分化とかなり密接な因果関係にあるようにみえる．

二次元分化の前後で bound auxin の量に著るしい差がみられるとはいえ，その質的差は少いことはこの因果関係を考察する上に重要なことである．さきに筆者らは，蛋白の質（ならびにその量）の変化が二次元分化を規定するのであろう．そしておそらく一次元生長期にはN代謝よりもC代謝が盛んであり，二次元生長期にはこの逆であろう，と結論した[4,5,6]．二次元分化と平行して見出された bound auxin の質，量の変化は，かかるN代謝，蛋白の変化の結果生じた二次的な生産物であろう．（上述の人為的一次元生長における結果もこのことを支持する）．

要するに，auxin はベニシダの二次元分化をおこさせる直接の因子とは考えられない．

本研究について，御指導・御鞭撻下さつた島村環，原田市太郎両博士にお礼申しあげます．

文　　献

1) Albaum, H.G., Amer. J. Bot. **25** : 37 (1938). 2) ———, Amer. J. Bot. **25** : 124 (1938). 3) 堀田康雄, 植雑, **73** : 69 (1960). 4) ————, 第 23 回日本植物学会大会 (1959). 5) Hotta, Y., and Osawa, S., Exptl. Cell Res. **15** : 85 (1958). 6) ———, ———, and Sakaki, S., Develop. Biol. **1** : 65 (1959). 7) Steeves, T. A., Georges, M., and Wetmore, R. H., Amer. J. Bot. **40** : 534 (1953). 8) Muir, R.M., and Hansch, C., Plant Physiol. **28** : 218 (1953). 9) Overbeek, J. van, Blondeau, R., and Horne, V., Amer. J. Bot. **42** : 205 (1955).

Summary

1. IAA and PAA added as growth substances in culture medium have respectively almost the same effect as the standard medium on the morphological differentiation of gametophyte of a fern, *Dryopteris erythrosora*.

2. No diffusible auxin is detected in all stages of gametophyte development examined.

3. Ether extractable auxin is detected only in later stages of gametophyte development when the meristematic region acts vigorously. In other stages, from spore to young gametophyte, ether extractable auxin is not detectable.

4. By the chromatographic analysis of bound auxin it is revealed that in the one-dimensional growth bound auxin is present as IAA and three growth promoting substances (Y_1, Y_2, Y_3) and in the two-dimensional growth as IAA, two growth promoting substances (Y_1, Y_2) and one growth inhibiting substance (Y_3').

5. From these results it may be concluded that changes of bound auxin are correlated solely to the two-dimensional differentiation.

Penicillium islandicum Sopp., NRRL 1175 の色素生成に対する Diphenylamine の影響

菊池正彦*・岡本好正*・林　孝三*

Masahiko KIKUCHI*, Yoshimasa OKAMOTO* and Kôzô HAYASHI*:
Effect of Diphenylamine on the Chromogenesis in *Penicillium islandicum* Sopp., NRRL 1175.

1959 年 10 月 2 日受付

Penicillium islandicum Sopp. の菌体色素として現在知られているものには，一群のアンスラキノン系色素と構造不明のエリスロスカイリンとがある．

さきに著者ら[1]は，菌株 NRRL 1175 の培養中における各色素成分の消長に基ずいてアンスラキノン系色素群とエリスロスカイリンとの間には直接的な生成的関連はないであろうと推論した．

これを裏書きする事実として，本菌株におけるエリスロカイリン生成がジフェニールアミンによって特異的に阻害されることがみられたので，この経過について報告する．なお，Pig–C, Pig–0.8 の構造についても，最近，柴田および著者らによって新たな知見が得られたので，この際これらの結果を合わせて，本菌における色素生成の経路についても考察してみたい．

この研究は東大薬学部の柴田教授の有益な示唆と助言を得つつ行なわれた．深く感謝の意を表する．

材料および方法

I．菌株：長尾研究所から分与された *P. islandicum* Sopp., NRRL 1175 を用いた．

II．培養基：

a)　完全液体培養基：蒸溜水 1 l 中に麦芽エキス (Bg. 1°)＋グルコース（3％）＋ペプトン (0.1％) を含むもの．

b)　最少液体培養基：Czapek-Dox 培養基（グルコース 3％）．

III．ジフェニールアミン (DPA) 原液：0.1M 濃度の無水アルコール溶液．

IV．培養：100 ml 容三角フラスコに各培養基 40 ml ずつを分注し，これらに，完全斜面培養 7～10 日の分生胞子を蒸溜水に懸濁したもの 0.2 ml ずつを接種し，所要の濃度になるように DPA 原液を添加して，27°±2° に保つ．

V．測定法：接種後適当な培養間隔で採取した菌体を前報[1]と同様の方法で処理し，ペーパークロマトグラフ法で色素成分を同定し，その有無によって DPA の影響を判定する．

供試菌株の色素成分について

前報[1]と同様の方法で，供試菌株は Chrysophanol, Pig–0.8, Erythroskyrin, Flavoskyrin, Skyrin, Oxyskyrin, Pig–C の各成分をもつことを確かめた．これらのうち，Pig–C, Pig–0.8 の構造については，つぎのような知見が加えられた．

Pig–C の構造：柴田ら[2,3]は，Pig–C をアルカリの存在下で，次亜硫酸ナトリウム ($Na_2S_2O_4$) で還元的に開裂するとき，ω–hydroxyemodin のみを生ずることから，おそらく Pig–C は ω–hydroxyemodin 2分子から成る Bis–体であろうと推定し，Thomson[4]の見解もまた同様であるから，Pig–C は Fig. 1. に示した構造に該当することはほぼ確実と見なされる．

Pig–0.8 の構造：柴田は，Pig–0.8 があるいは Emodin ではないかと考えて，著者らに，その同定を求めた．著者らは Emodin の結晶標品を対照として，供試菌株のアセトン抽出液を用いて，ペーパークロマトグラフ法（方法は前報[1]参照）で両色素の一致を確認した（表 1）．

なお，クロマトグラム上の Pig–0.8 相当区分を

* 東京教育大学理学部植物学教室 Botanical Institute, Faculty of Science, Tokyo University of Education, Ohtsuka, Tokyo, Japan.

Table 1. Paper chromatographic comparison of Pig–0.8 with authentic sample of emodin. (Solvent: upper layer of acetone/benzine/water = 5:5:3.5, paper: Toyo Roshi No. 3, temp.: 24°.)

	Rf value	Color on paper	Reaction with methanolic Mg–acetate on paper	
			at room temp.	on heating
Pig–0.8 from acetone extract of the test fungus	0.80	Orange	Orange–red	Pink
Emodin (authentic)	0.80	Orange	Orange–red	Pink

溶出し，Emodin の結晶標品とともに混合クロマトグラフィを行なったが，両者の分離は見られなかった．したがって，Pig–0.8 を Emodin (Fig. 1 参照) と見なしてさしつかえないと考える．

培養実験および結果

ジフェニールアミン (DPA) の影響

下記の所見はいずれも 3 回反覆した実験の結果による．

〔実験 1〕 まず，液体最少培地に DPA 濃度がそれぞれ 5×10^{-4} M, 5×10^{-5} M, 5×10^{-6} M になるように DPA 原液を添加する (各培地に含まれるアル

Table 2. Effect of DPA added to the complete medium on the chromogenesis

Days after inoculation	Control: Complete medium (C.M.) without DPA (pH 6.2)		DPA (5×10^{-5} M) in C.M. Final conc. of EtOH: 0.05% (pH 6.2)	
	Observation	Pigment formation	Observation	Pigment formation
3	Colonies cover the whole surface of the medium. Mycelia: grayish orange–red.	Formation of anthraquinone pigments is complete.	Colonies cover the whole surface of the medium. Mycelia: slightly grayish yellow.	
4	Mycelia: orange-red. Spore formation occurs.	Erythroskyrin appears.	Mycelia: orange.	Formation of anthraquinone pigments is complete.
5			Mycelia: reddish orange.	
6			Spore formation occurs.	Erythroskyrin appears.
7				
9				
10				
16				

コールの最終濃度はそれぞれ 0.5, 0.05, 0.005%となる). なお 0.5% にアルコールを含む液体最少培地を対照とし, これらの培地へほぼ等量の胞子を接種し, 菌の生育状況と色素生成とをしらべた.

その結果, DPA はアンスラキノン系色素の生成にはほとんど影響を与えないが, Erythroskyrin の生成を著るしく(対照に比べて約1週間) 遅らせ, 最大の作用濃度は 5×10^{-4} M と 5×10^{-5} M の間にあることが観察された. また, アルコール濃度 0.5%では菌の生育, 色素生成はほとんど影響されないことがわかった.

また, 前報[1]で述べたように, 培地の pH は一旦酸性に傾き, ついでアルカリ性に戻ることも確められた.

〔実験2〕 上記の実験において, DPA は 5×10^{-4} M~5×10^{-5} M で Erythroskyrin 生成を顕著に阻害することがみられたので, この濃度範囲をさらに細分してしらべた.

すなわち, 最少液体培地, 完全液体培地の2者を用意し, DPA をそれぞれ 5×10^{-5} M, 8×10^{-5} M, 1×10^{-4} M, 2.5×10^{-4} M に添加し, これらの培地上での菌の生育, 色素生成の状態をしらべた. この際 DPA を含まない各培地そのままを対照とした*.

i) 完全培地に DPA を添加した場合

結果は表2に示したとおりで, つぎのように要約される.

生育: DPA 5×10^{-5} M 添加のものでは, 対照に

* 実験1により, 0.5% 以下のアルコールは影響がない. Jensen *et al.*[5] もまた *Rhodospirillum* の生育とカロチノイド形成は 0.25% エタノールで影響されないことを報じている.

in the mycelia of *P. islandicum* Sopp., NRRL 1175 during culture.

DPA (8×10^{-5} M) in C.M. Final conc. of EtOH: 0.1% (pH 6.2)		DPA (1×10^{-4} M) in C.M. Final conc. of EtOH: 0.1% (pH 6.2)		DPA (2.5×10^{-4} M) in C.M. Final conc. of EtOH: 0.1% (pH 6.2)	
Observation	Pigment formation	Observation	Pigment formation	Observation	Pigment formation
Colonies (all of them: 1~1.5mm. in diam.) develop.		Colonies (all of them: 11.5mm. in diam.) develop.			
Colonies cover the whole surface of the medium. Mycelia: pale orange.	Formation of anthraquinone pigments is complete.	Colonies cover the whole surface of the medium. Mycelia: slightly grayish yellow.			
Mycelia: orange color deepens.		Mycelia: orange color deepens.	Formation of anthraquinone pigments is complete.		
Mycelia: orange-red.					
Mycelia: reddish orange.		Mycelia: orange-red.			
				Colonies (all of them: 1~2mm. in diam.) cover the surface of the medium.	
Mycelia: brownish orange-red. Spore formation occurs.	Erythroskyrin appears.	Mycelia: brownish orange-red. Spore formation occurs.	Erythroskyrin appears.		
				Colonies (all of them: 2~3mm. in diam.) develop. Mycelia: orange to brownish orange	Formation of anthraquinone pigments is complete.

比べて生育は僅かに劣り，8×10^{-5} M 添加では前2者に比べて1日ぐらいのおくれが見られる．

DPA 1×10^{-4} M 添加のものは，8×10^{-5} M のものより僅かに劣る程度である．しかし DPA 2.5×10^{-4} M 添加では，生育は著るしく不良となり，接種後 16 日でも径 1〜2 mm の集落が孤立的に生ずるのみで液面を膜状に覆うことはない．

アンスラキノン系色素の生成：DPA 5×10^{-5} M, 8×10^{-5} M 添加のものは，対照に比べて，色素の出現がやや（1日位）おくれる．DPA 1×10^{-4} M 添加では，前2者よりもさらに1日位おくれる．しかし DPA 2.5×10^{-4} M 添加のものでは 12 日位の遅れを生ずる．

エリスロスカイリンの生成：DPA 5×10^{-5} M 添加のものは，対照に比べて色素の出現が 2〜3 日おくれ，その量も少ない．8×10^{-5} M, 1×10^{-4} M 添加のものでは1週間位おくれる．しかし，2.5×10^{-4} M 添加のものでは2週間後でも色素形成は見られない．

ii） 最少培地に DPA を添加した場合

この場合においても，菌の生育および色素生成に対する DPA の影響は，完全培地上でみられた結果（表2）とほとんど同様である．（結果の表記は省略）．ただし DPA 5×10^{-5} M の場合，Erythroskyrin の出現については完全培地上に比べて，いっそう顕著な遅れが見られる．

総括および考察

上記の結果から，DPA は Erythroskyrin の生成に著るしく影響することがわかる．すなわち，DPA 5×10^{-5} M, 8×10^{-5} M, 1×10^{-4} M の3者では，菌の生育およびアンスラキノン系色素の生成には見るべき阻害はないが，これに反して Erythroskyrin の生成は約1週間おくれ，量的にも極めて少ない．

DPA 2.5×10^{-4} M では，生育は著るしく抑制され，アンスラキノン系色素の出現もおくれるが，各成分には異常がない．他方，Erythroskyrin の生成は完全に阻害される．

さきに最少培地上での継代培養（前報[1]：表 2—4）によって，アンスラキノン系色素群のうち Flavoskyrin, Emodin (Pig-0.8) の生成能力が消失した後にもなお Erythroskyrin の生成が観察されたが，今回 DPA によって，これらアンスラキノン系色素の生成はほとんど影響されずに Erythroskyrin の生成だけが阻害されたことは注目に値する．

DPA の色素生成への影響については，最初 Kahrasch *et al.*[6] (1936) が細菌，糸状菌について報告しているが，その中には *Rhodospirillum*[7], *Micrococcus*[8], *Rhodotorula*[9] などのカロチノイド生産菌が含まれている．その後，*Mycobacterium*[10,11], *Phycomyces*[12,13,17], *Rhodospirillum*[5,14], *Rhodopseudomonas*[15], *Chlorobium*[16], *Allomyces*[18], *Neurospora*[19] などについて，カロチノイド生成が DPA によって特異的に抑制されることが報ぜられた．DPA のこのような抑制機構については，目下のところはカロチノイドの共役二重結合形成のための脱水素反応の阻害であろうと推論されているにすぎない．

ともかくも，Erythroskyrin の生成が DPA によって特異的に阻害されることからすると，この色素の生成機構はカロチノイドのそれと共通なものを含むことが暗示される．

この機会に，著者らの実験結果とアンスラキノン系色素の生合成仮説との関連について若干考察してみたい．

柴田[3]は Collie[20], Birch and Donovan[21], Robinson[22] らの 'acetate theory' に基ずいて，*Penicillium* 菌におけるアンスラキノン系色素相互間の生成的関連を推論し，色素形成の初期段階に Proto-flavoskyrin, Proto-rugulosin の2つの中間体を仮定して，Fig. 1 のような生合成経路を考えている．

すなわち，8個の acetate units が head to tail につながり，環式化によって Proto-flavoskyrin が生じ，その coupling によって Proto-rugulosin が形成され，さらに $2H_2$ を失って Skyrin へ移行し，Skyrin の 7′-CH_3 の酸化によって Oxyskyrin を経て Pig-C が形成されると考え，この経路が本菌におけるアンスラキノン系色素生成の主幹であり，この経路から溢れたものが一つの副経路

$$\text{Proto-flavoskyrin} \begin{cases} \nearrow \text{Chrysophanol} \\ \searrow \text{Flavoskyrin} \rightarrow \text{Emodin} \end{cases}$$

を経て，Chrysophanol, Flavoskyrin, Emodin が形成されるであろうと考えた (Fig. 1)．

著者らは，この仮説にかかわりなく，もっぱら最

Fig. 1. Shibata's hypothetial biosynthetic scheme of pigment formation in the mycelia of *P. islandicum* Sopp., NRRL 1175.

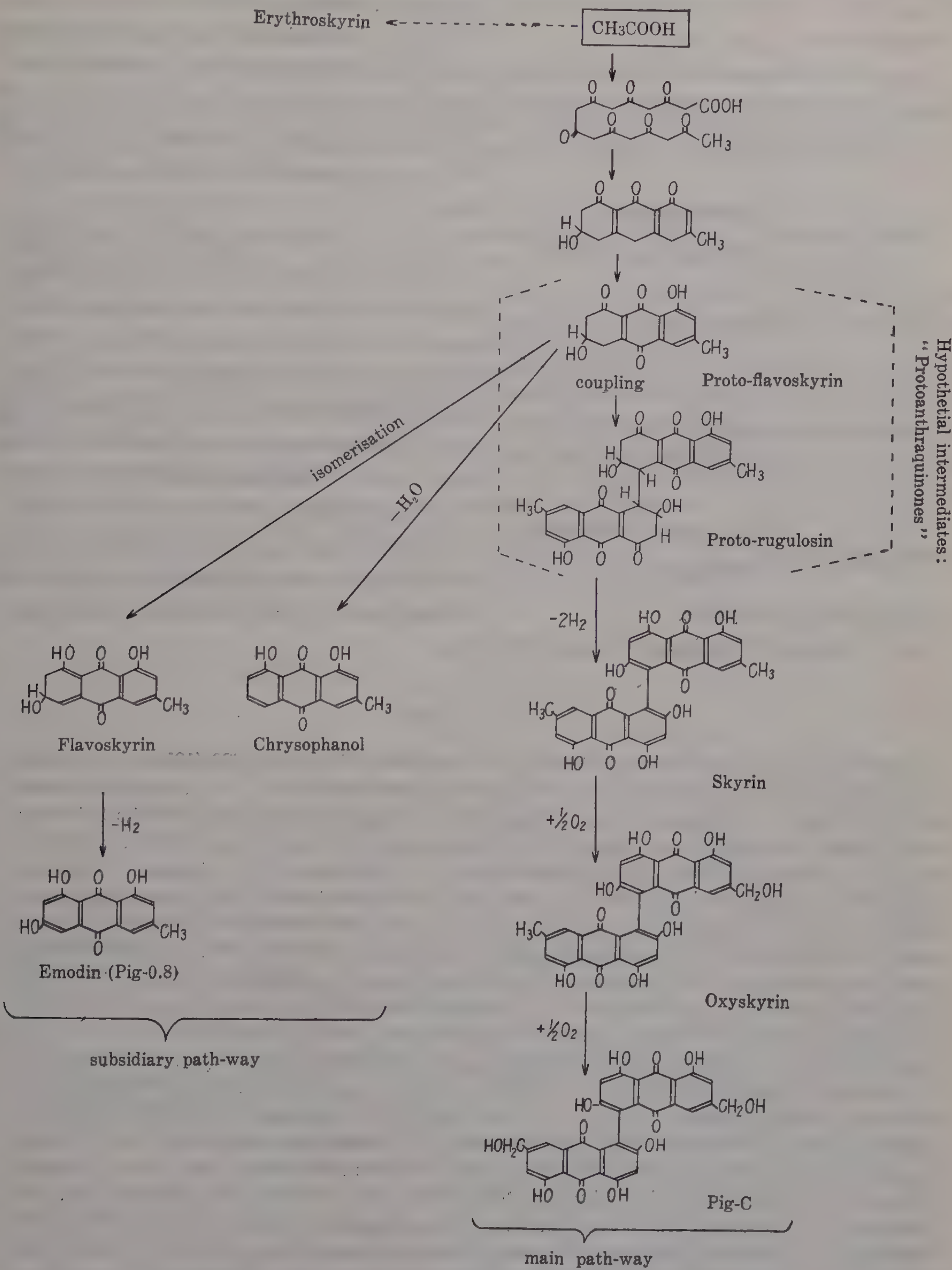


Fig. 1

少培地上での継代培養（前報：表 3，4），および培養時における色素形成の順位を追跡した結果（前報[1]）：表 5—8），上記の柴田説の主要経路に対しては実験的裏付けを与えることができた．

しかし，Proto-flavoskyrin から Chrysophanol, Flavoskyrin, Emodin に至る副経路については，なお若干の問題を残している．柴田は表 1 に示したように

$$\text{Proto-flavoskyrin} \begin{cases} \nearrow \text{Chrysophanol} \\ \searrow \text{Flavoskyrin} \rightarrow \text{Emodin} \end{cases}$$

の経路を推定しており，著者らの最少培地上での培養実験において Emodin (Pig–0.8) と Flavoskyrin の消失，およびその形成能力回復の結果（前報[1]）：表 3, 4）は，一応は柴田の考えを支持するとも見られるが，培養中に Emodin (Pig–0.8) が常に Flavoskyrin より早く出現する事実（前報[1]）：表 8）からすると

$$\text{Proto-flavoskyrin} \begin{cases} \nearrow \text{Chrysophanol} \rightarrow \text{Emodin} \\ \searrow \text{Flavoskyrin} \end{cases}$$

の経路が妥当のように思われる．

しかし，この場合 Chrysophanol→Emodin の過程には *meta*-hydroxylation を必要とする点に無理がある．他方，高等植物で，しばしば Chrysophanol と Emodin とが共存する事実[23]）を考え合わせると，Chrysophanol, Emodin, Flavoskyrin の 3 者は共通の precursor（たとえば柴田の Proto-flavoskyrin）から平行的に生成するか，それともまた Emodin に別個の precursor を当てるか，この辺の事情については今後の研究にまたなければならない．

アンスラキノン色素の生合成においては，中間体について，なお問題は残されているが，acetate から出発するという見解については議論の余地はないようである．すなわち，最近 Birch *et al.*[24]），Gartenbeck[25]）は放射性酢酸 (1–C^{14}) を用いて放射性の Helminthosporin (4, 5, 8–Trihydroxy–2–methyl anthraquinone) および Emodin の生合成を報じ，柴田ら[23]）も同様にして Skyrin, Rugulosin への incorporation を確認しているからである．なお今回の DPA による生成阻害の実験から Erythroskyrin がカロチノイド様物質という見方も acetate theory の一環として矛盾するものではない．

文　　献

1) Hayashi, K., Kikuchi M., and Okamto, Y., Bot. Mag. Tokyo **72** : 220 (1958). 2) Shibata, S., Takido, M., and Nakajima, T., Phar. Bull. **3** : 286 (1955). 3) ——, Kagaku (Japan) **26** : 391 (1956). 4) Thomson, R. H., Naturally occurring quinones. Butterworths Scientific Pub. London, 241 (1957). 5) Jensen, S. L., Cohen-Bazire, G., Nakayama, T.O.M., and Stanier, R. Y., Biochim. Biophys. Acta **29** : 477 (1958). 6) Kahrasch, M. S., Conway, E. A., and Bloom, W., J. Bact. **32** : 533 (1956). 7) van Niel, C. B., and Smith, J. H. C., Arch. Microbiol. **6** : 219 (1935). 8) Chargaff, E., and Lederer, E., Ann. Inst. Pasteur **54** : 383 (1935); Chem. Abst. **30** : 5252 (1936). 9) Lederer, E., Bull. Soc. Chim. biol. Paris **20** : 611 (1938); Chem. Abst. **32** : 7500 (1938). 10) Turian, G., Helv. Chim. Acta. **33** : 1988 (1950); Chem. Abst. **45** : 3021 (1951). 11) ——, and Haxo, F., J. Bact. **63** : 690 (1952); Chem. Abst. **46** : 7175 (1952). 12) Goodwin, T. W., Biochem. J. **50** : 550 (1952); Chem. Abst. **46** : 4053 (1952). 13) ——, Jamikorn, M., and Willmer, J. S., Biochem. J. **53** : 531 (1953). 14) ——, and Osman, H. G., Biochem. J. **53** : 541 (1953); **56** : 222 (1954). 15) ——, Land, D. G., and Osman, H. G., Biochem. J. **59** : 491 (1955). 16) ——, and Land, D. G., Biochem. J. **62** : 553 (1960). 17) Varma, T. N. R., Chichester, C. O., and Mackiney, G., Nature **183** : 188 (1959). 18) Turian, G., and Haxo, F., Bot. Gaz. **115** : 254 (1954); Biol. Abst. **29** : 1226 (1955). 19) ——, Physiol. Plantarum **10** : 667 (1957); Biol. Abst. **32** : 6451 (1958). 20) Collie, J. N., J. Chem. Soc. **91** : 1806 (1907). 21) Birch, A. J., and Donovan, F. W., Austral. J. Chem. **6** : 361 (1953). 22) Robinson, R., Structural relations of natural products. Oxford Univ. Press, 10 (1955). 23) Shibata, S., and Takido, M., J. Pharm. Soc. Japan **72** : 1311 (1952); Tsukida, K., Suzuki, N., and Yokota, M., J. Pharm. Soc. **74** : 224 (1954); Tsukida, K., and Yoneshige, M., J. Pharm. Soc. **74** : 379 (1954); Tsukida, K., Yoneshige, M., and Tsujioka, J., J. Pharm. Soc. **74** : 382 (1954); Tsukida, K., J. Pharm. Soc. **74** : 386, 394, 398, 401 (1954). 24) Birch, A. J., Ryan,

A. J., and Smith, H., J. Chem. Soc. 4773 (1958). 25) Gartenbeck, S., Acta Chem. Skand. **12** : 1211 (1958). 26) Shibata, S., Private communication.

Summary

1) The effect of diphenylamine (DPA) added to the culture media upon the chromogenesis of *Penicillium islandicum* Sopp., NRRL 1175, was investigated, and the results are shown in Table 2. At lower concentrations below 1×10^{-4} M, DPA did not show any significant effect on the mycelial growth and the formation of erythroskyrin was delayed nearly a week in comparison with the control.

2) At the concentration of 2.5×10^{-4} M DPA, the growth of the fungus as well as the formation of anthraquinone pigments was apparently reduced, but any of the component anthraquinones was not lost. However, the formation of erythroskyrin was completely abolished in this case.

3) Since DPA has been shown to be specific inhibitor for the carotenogenesis in general (Goodwin *et al.*), erythroskyrin seems to be a carotenoid-like substance.

4) As regards the unknown pigments described in the preceding paper[1]), pig–0.8 was identified as emodin (*cf.* Fig. 1) by paper chromatography (Tab. 1), and pig–C has been made plausible by Shibata *et al.*[2,3]) to have the structure of 4, 5, 7, 4′, 5′, 7′–hexahydroxy–2, 2′–dihydroxymethly–bis (1–1′)–anthraquinone (*cf.* the structural formula in Fig. 1).

5) In the light of these and previous results[1]) of our experiments, biosynthetic interrelationship of the pigments concerned was discussed. In consequence, it was shown that Shibata's hypothetial biosynthetic scheme (Fig. 1) is consistent in its essential feature with our experimental findings, but not in subsidiary pathway involving chrysophanol, flavoskyrin and emodin. This awaits further studies.

ヒヤシンスの小仁染色体について

辰野誠次*・瀬川道治*

Seizi TATUNO* und Michiharu SEGAWA*: Über die Nukleolinus-Chromosomen von *Hyacinthus orientalis*.

1959 年 10 月 16 日受付

さきに辰野 (1954 a[1]), b[2])) は苔類の一種ケゼニゴケ (*Dumortiera hirsuta*) の小形な染色体 *m* が heterochromatisch で，その染色体の一部が休止，前期および後期の核で仁内に入り小仁 (Nukleolinus) を作ることを発見し，このような小仁を作る染色体を小仁染色体 (Nukleolinus-Chromosomen) と呼んだ．その後蘚苔類では，蘚苔類に普遍的な *m* および付随体染色体は付随体が小仁を作り，小仁染色体であることがわかった (Tatuno u. Segawa 1955[3]), Tatuno 1956[4]), '57[5]), Yano 1957 a[6]), b[7]), c[8])．しかるに，顕花植物ではいわゆる仁染色体はいずれも仁の表面に付着し，小仁形成の明らかな報告はほとんどなく，僅かに最近征矢野氏 (1958[9])) が *Allium cepa* で，付随体起源の小仁の見られたことを簡単に記述されているに過ぎない．そこで筆者らは顕花植物における小仁染色体の発見につとめているが，今その一例としてヒヤミンスの小仁染色体について報告する．

筆者らが観察にもちいたヒヤシンスは市販の 2n = 16 の染色体 (Fig. 5, 6) を持った 1 品種である．観察は根端を 0.004 M の 8 オキシキノリンで 3 時間前処理し，それを Feulgen 反応および酢酸オルセイン染色したものによった．

休止核の仁内には，Fig. 1, 2 に示すごとく，明らかに Feulgen 陽性の 2 個の小仁が見られる．その形は普通球状であるが，まれにその一部がほぐれて，繊維状を示すこともある．しかし，蘚苔類の場合のごとく，その細い繊維構造が仁表まで達しているか否かは未だ明らかでない．この小仁は Feulgen 陽性を示す点から染色体起源であって，蘚苔類の *Asterella*, *Plagiochasma* 等 (Tatuno 1956[4])) の付随体染色体と同様，本種の前期 (Fig. 3, 4) に見られる 2 個の付随体染色体の付随体に由来するものである．

一般に 2n = 16 のヒヤシンスでは 1 組の染色体の 8 個のうち 2 個が小形で Darlington *et al.* (1951[10])) はこれを S_1, S_2 で示しており，S_1 は S_2 よりも狭窄の位置が中央に近い．筆者らの観察した付随体染色体は明らかにその S_1 に相当し，付随体はその短腕の端に付着している．なお興味あることは，この S_1 の付随体は中期 (Fig. 5, 6) においては Feulgen 反応およびオルセイン染色においても染色されず認められないことである．したがって，Darlington 氏らも未だ本種には付随体染色体を報告していない．すなわち，この付随体は休止期においてはいわゆる positive Heteropyknose を示して小仁を作り，前期から中期に到って，染色力が反転し，negative Heteropyknose を示すものと考えられる．これは蘚苔類の *Targionia hypophylla* などの小仁染色体の場合と同様である (Tatuno 1956[4]))．

* 広島大学理学部植物学教室 Botanisches Institut der Universität zu Hiroshima, Hiroshima, Japan.

Fig. 1–6: Chromosomen der Wurzelspitze von *Hyacinthus orientalis*. 1, 2: Ruhekern, wobei die zwei Nukleolini immer im einen Nukleolus erkennbar sind. 3, 4: Prophase, zwei Satelliten-Chromosomen sind sichtbar. 5, 6: Metaphase, $2n = 16$. Vergr. 1500.

文　　献

1) 辰野誠次，植雑 **67**: 36 (1954a). 2) Tatuno, S., J. Sci. Hiroshima Univ. ser. B, div. 2, **6**: 251 (1954b). 3) ———, u. Segawa, M., ibid. **7**: 1 (1955). 4) ———, ibid. **7**: 119 (1956). 5) ———, ibid. **8**: 81 (1957). 6) Yano, K., Memo. Fact. Education, Niigata Univ. **6**: 1 (1957a). 7) ———, Memo. Takata Branch Niigata Univ. **1**: 85 (1957b). 8) ———, ibid. **1**: 129 (1957c). 9) 征矢野芳孝，染色体 **37–38**: 1282 (1958). 10) Darlington, C. D., Hair, I. B., and Hurcombe, R., Heredity **5**: 233 (1951).

Zusammenfassung

Bei einer Gartenvarietät ($2n = 16$) von *Hyacinthus orientalis* könnten wir Nukleolinus-Chromosomen entdecken. Die zwei Nukleolini in jedem Nukleolus der Ruhekerne stammen aus den Satelliten der zwei Satelliten-Chromosomen. Die Satelliten sind in Metaphase im allgemeinen unsichtbar, da sie dabei negative Heteropyknose zeigen.

本　会　記　事

役　員　変　更

この度東北支部選出の評議員神保忠男氏の代りとして，吉岡邦二氏が評議員になられました。

支　部　通　信

関東支部

昭和 34 年度関東支部大会（4 月 5 日，お茶の水大学において）

竹内亮: Manchuria のスミレ属についての二三の知見，鈴木貞雄: 関東，東北地方産ミヤコザサ類の分類，百瀬静男: いわゆる Leptosporangiatae の系統起源群について，沢村正五: 各種除草剤の作用に対する生体細胞的研究 II，竹村英一: ヒガンバナ属 (*Lycoris*) の人工雑種について III，石川茂雄，中川　篤，藤伊　正: Gibberellin 処理による種子の発芽機作の解析 I，石川茂雄，藤伊　正: Gibberellin 処理による種子の発芽機作の解析 II，田中実，三輪知雄: シダ植物における γ-ヒドロキシ-γ-メチルグルタミン酸の分布，延原　肇: 繁殖型を異にする海浜植物の舌状丘形成，斎藤規夫，三井清司，林　孝三: 薬用サフランの花の色素 Hyacin＝Delphin の証明，大槻虎男: リビリイモおよびヤマコンニャクの貯蔵多糖類について，広川秀夫: 大腸菌ペニシリンプロトプラストから桿菌細胞への復帰様式

中部支部

第 58 回例会（2 月 6 日，愛知県立女子大学において）

岩塚　寿: いおう細菌の CO_2 固定，川中建雄: 私の見る上代の植物観

九州支部

第 58 回例会（2 月 13 日，九大・理において）

沢田武男，檜垣正浩，吉田忠生: 津屋崎周辺のホンダワラ類群落，下鶴大輔: コンゴー旅行談

なお九州支部の役員とし，つぎの方がきまりました．

(1) 支部長: 細川隆英，(2) 委員: 細川隆英，瀬川宗吉，野口　彰，千葉保胤，芳賀　忞，(3) 地区委員: 村山宅美（佐賀），外山三郎（長崎），服部新佐（宮崎），鈴木時夫（大分），山根銀五郎（鹿児島）

Studies on the Dehydration Resistance of Higher Plants II
Theoretical Consideration of Dehydration Resistance*

by Tadayoshi TAZAKI**

Received November 6, 1959

In a previous paper[1]) the result of experiments was mentioned for determining measures related to the dehydration resistance of mulberry plant (*Morus alba*). In this paper will be introduced some mathematical formulae concerning the water economy and then the dehydration resistance, of higher plants to begin with.

1. Fundamental equation of water economy and dehydration resistance

At first the water economy of higher plant in normal condition will be considered. The most general expression for the water economy of a plant or a plant part is,

$$\Delta \boldsymbol{W}=\boldsymbol{W}_t-\boldsymbol{W}_o=\int_o^t \boldsymbol{A}\ dt-\int_o^t \boldsymbol{T}\ dt, \tag{1}$$

where $\boldsymbol{W}_o$ and $\boldsymbol{W}_t$ are the water amount at the beginning and after the time interval of t, and $\boldsymbol{T}$, $\boldsymbol{A}$ are the transpiration and water absorption for unit time.

In a detached plant part the first term in the right side of equation (1) is zero, as no water supply occurs after detaching and if its water content falls to its lethal water content after t from detaching, $\boldsymbol{W}_o-\boldsymbol{W}_t$ will turn out to be lethal deficit ($\boldsymbol{D}$), corresponding to the water amount that can be allowed to transpire before the plant part is killed by dehydration (Pisek and Berger's "verfügbares Wasser"[2]). Then equation (1) can be rewritten for this special case of dehydration resistance as,

$$\boldsymbol{D}=\int_o^t \boldsymbol{T}\ dt\ . \tag{2}$$

By the definition of dehydration resistance mentioned in a previous paper[1]), i. e., the time required to kill the plant part after detaching, it is clear that the solution of equation (2) for t namely gives the value of dehydration resistance ($\boldsymbol{t}$). Next, t and $\boldsymbol{t}$ were expressed as minutes in order to cover the most susceptible cases of dehydration resistance less than an hour, but it is advisable to use hours or days in more resistant cases. For the concrete determination of $\boldsymbol{t}$, $\boldsymbol{T}$ must be expressed as the function of t, and $\boldsymbol{D}$ and $\boldsymbol{T}$ must be substituted by several measures concerning the water relation of a plant part. In the following the author will put forward two analyses based on equation (2), general and special. General analysis can be applicable to every case of dehydration resistance including the case in which leaf area can hardly be determined, while special analysis can only be applicable to leaves whose area can readily be determined. We shall begin by examining this special analysis focussing on mulberry plant.

---

* Reported at the 18th General Meeting of the Botanical Society of Japan (1953).

** Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan.

2. Special analysis

Let us consider the leaf area of $1\,cm^2$. and express both side of the equation (2) in mg. Lethal deficit (***D***) is usually expressed as the percentage on an oven dry basis (D), so the left side of the equation (2), i. e., lethal deficit in mg. of unit area of leaf (cm^2.) will be, $(D/100)\times 1000\times(1/1000)\times M$ or $(DM/100)$ mg., where M is the dry weight in mg. of leaf for unit area. As for the right side of the equation, it may be preferable to express the transpiration amount in relative value or relative transpiration (T_r)[1]) in order to be freed from the influence of humidity. To avoid the margin effect due to the area and shape of evaporating surface the value of T_r should be the ratio, the transpiration of a leaf to the evaporation of leaf-shaped evaporimeter, i. e., a moistened filter paper of leaf shape with one or both evaporating surface, during the same time interval and at the same humidity. Thus,

$$T_r=\frac{\boldsymbol{T}}{Ed}\times 100 \quad (\%) \qquad \text{or} \quad \boldsymbol{T}=\frac{T_r}{100}\times Ed, \tag{3}$$

where E and T are, respectively, the amount of evaporation for a leaf-shaped evaporimeter, mg./cm^2./hr./1 mm. Hg., and the amount of transpiration for a leaf, mg./cm^2./hr./d mm. Hg. Putting the value of ***T*** and ***D*** into equation (2) we get

$$\frac{DM}{100}=\int_o^t \frac{T_r}{100}\times\frac{1}{60}\times Ed\ dt,$$

$$\text{or} \quad DM=\frac{Ed}{60}\int_o^t T_r\ dt. \tag{4}$$

Now we have come to a position to express T_r as the function of t, for which following empirical formula, equation (5), was applied to the time-T_r curves obtained by the author in mulberry plant and in *Quercus myrsinaefolia* (unpublished data).

$$T_r=A'e^{-k't}+C' \tag{5}$$

In this equation k' is the tendency of T_r decrease, A' the amount of stomatal T_r immediately after detaching leaves and C' the final amount of T_r, i. e., in most cases the amount of relative cuticular transpiration after stomatal closure (T_{rc}), though the latter two amounts never coincide in some exceptional cases such as in "dull" leaves[1]) of mulberry plant whose stomata remain open until the death by dehydration.

Substituting the T_r value of equation (5) into equation (4) we obtain,

$$\frac{60DM}{Ed}=\int_o^t (A'e^{-k't}+C')dt.$$

Integration of the right side gives,

$$ln\left\{C't+\left(\frac{A'}{k'}-\frac{60DM}{Ed}\right)\right\}=ln\,\frac{A'}{k'}-k't, \quad \text{or}$$

$$\log_{10}\left\{C't+\left(\frac{A'}{k'}-\frac{60DM}{Ed}\right)\right\}=\log_{10}\frac{A'}{k'}-0.4343k't. \tag{6}$$

The value of dehydration resistance (***t***) can be obtained by the graphical solution of equation (6). The solution, however, can be simplified in some cases as will be mentioned in the following sections.

3. Discussion for special analysis focussing on mulberry plant

In this section the determination of dehydration resistance by special analysis will be discussed focussing on mulberry plant. At first an example was cited of the time trend of T_r after detaching leaves measured in August, 1952 for a "summer cut" shoot of Kairyô-nezumigeshi, a form of mulberries. In Fig. 1 are shown the time-T_r curves after detaching leaves, in which equation (5) was applied for different values of k', a) and d) from the results of actual measurement, and b) and c) for supposed cases. The values of A' and C' were, respectively, 100 and 10 in these samples. In the curve of "dull" leaf with $k'=0.0230$, the curve was applied only to the initial stage. The graphical solution of equation (6) based on these curves was illustrated in Fig. 2, in which A', D, M, and E were, respectively, 100, 110, 5 and 1. For the calculation of $\boldsymbol{t}$ the value of d was always assumed to be 10 mm. Hg. Dehydration resistance ($\boldsymbol{t}$) can be obtained from the t-coordinate of the intersection point of left side (y_1) and right side (y_2) functions. But, as will be noticed from a) in Fig. 2 the curve y_1 runs perpendicularly to t-axis and is almost rectilinear in the negative area of ordinate and in the neighbourhood of intersecting point with t-axis, in other words, if the t value obtained by solving the equation, $y_1=0$, is larger than that by solving the equation, $y_2=0$, the value of $\boldsymbol{t}$ will be able to be

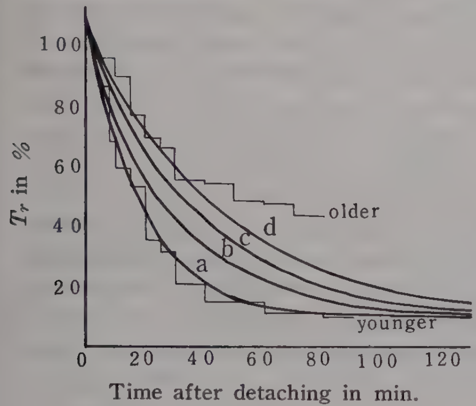


Fig. 1. Time-T_r curves in "summer cut" mulberry plant in summer. Stepwise lines are the measured transpiration in normal (younger) and "dull" (older) leaves, and four curves are drawn by equation (5) for k' values, respectively, 0.0575 (**a**), 0.0384 (**b**), 0.0288 (**c**) and 0.0230 (**d**); $A'=100$ and $C'=10$.

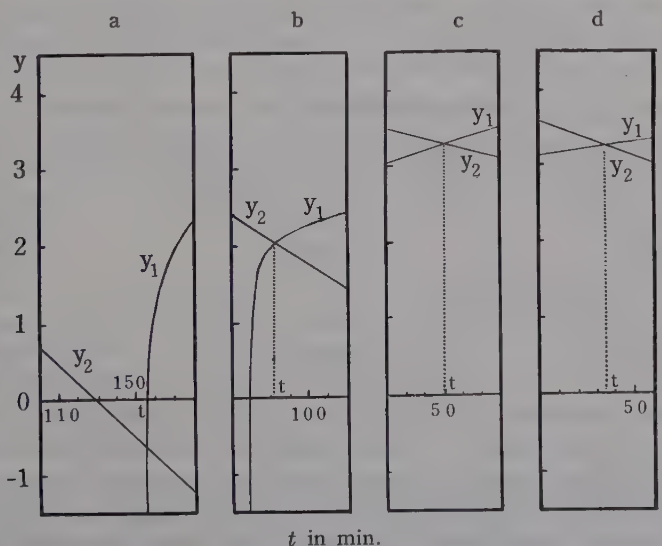


Fig. 2. Graphical solution by equation (6) of the dehydration resistance ($\boldsymbol{t}$) of mulberry leaves based on four curves (a, b, c and d in Fig. 1). The values of $\boldsymbol{t}$ for a, b, c, and d were, respectively, 156, 82, 52 and 35 min. Further explanation was written in the text.

obtained only by solving $y_1=0$ irrespective of y_2 function. Now, the value of t obtained from $y_1=0$ is practically the same with that from $y_1=-\infty$, so we can substitute this equation for $y_1=0$, thus

$$\log_{10}\left(C't+\frac{A'}{k'}-\frac{60DM}{Ed}\right)=-\infty,$$

or

$$t=\frac{1}{C'}\left(\frac{60DM}{Ed}-\frac{A'}{k'}\right). \tag{7}$$

The condition for this solution above mentioned will be expressed as a following inequality if we also substitute $y_1=-\infty$ for $y_1=0$.

$$\frac{1}{C'}\left(\frac{60DM}{Ed}-\frac{A'}{k'}\right)\geqq\frac{1}{0.4343k'}\log_{10}\frac{A'}{k'} \tag{8}$$

As is noticed from inequality (8) the left side becomes smaller and, on the contrary, the right side becomes larger in accordance with the value of k' becoming smaller when all measures other than k' are constant, resulting at last in the failure of this inequality. So equation (7) holds in cases save for those in which the value of k' is small. The failure of this inequality has also bearing on other measures in the inequality, the detailed discussion of which will be mentioned in another report of this investigation.

In still other cases in which dehydration resistance is so large that the course of time trend in T_r can be neglected, it suffice to put C' instead of T_r in equation (4), the solution of which gives the value of t as,

$$t=\frac{60DM}{C'Ed.} \tag{9}$$

It is clear that the omission in equation (7) of the term A'/k', the measures concerned with the course of diminishing transpiration, gives namely equation (9) and that t values obtained from equation (9) are always larger than those from equation (7) by $A'/C'k'$. In less resistant cases the value of $A'/C'k'$ becomes so large that the application of equation (9) results in ill defined values and in this case we must resort to equation (7) for the determination of dehydration resistance.

Table 1. Calculated values of dehydration resistance in mulberry leaves by three equations

Calculated by equation	Younger leaves (normal)	Older leaves ("dull")
(6)	156min.	35min.
(7)	156	–134
(9)	300	300

Application of equations (6), (7) and (9) to normal (a) and "dull" (d) leaf in Fig. 1 resulted in the figures in Tab. 1. from which it is obvious that equation (9) can never be applied to both kinds of leaves and that equation (7) can only be applied to normal leaves. The limitation of the applicability of equation (7) must be examined from various aspects, but at least it is certain that there exist some cases in which equation (7) can not be applied in the dehydration resistance of mulberry plant.

As will be noticed from equation (7) the product, DM, has much bearing on the value of dehydration resistance (t). The relationships between lethal deficit (D) and

areal weight (M) in several plants were illustrated in Fig. 3 by the data of Pisek and Berger[2]) for European plants. The point for mulberry plant was dotted by the data of the author. The points on hyperbolas in the figure show equal values of DM, i. e., 7000, 2000, 1000, 500 and 300. The mightiest of all in dehydration resistance at least concerning DM was *Sedum*, a succulent species, with large values of both D, and M, while *Veronica* was resistant due to the large values of D, and *Picea*, *Rhododendron* and *Pinus silvestris* were also resistant owing to the large value of M. Other species of deciduous trees and herbs including our *Morus* plant were rather susceptible with the values of DM less than 1000, though the values of D and M were widely different by species.

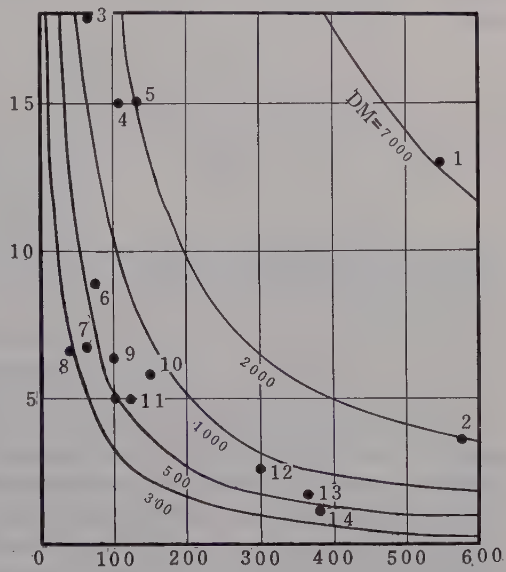


Fig. 3. The relationships between D and M in several European plants adapted from the data of Pisek and Berger[2]). 1. *Sedum maximum*, 2. *Veronica baccabunga*, 3. *Picea excelsa*, 4. *Rhododendron ferrugineum*, 5. *Pinus silvestris*, 6. *Quercus robur*, 7. *Corylus avellana*, 8. *Fagus silvatica*, 9. *Betula verucosa*, 10. *Hedera helix*, 11. *Morus alba*, (by T. Tazaki), 12. *Asarum europeum*, 13. *Stellaria nemorum*, 14. *Impatiens noli-tangere*. Ord. M, Absc. D.

4. General analysis

In general analysis it is advisable to deal with equation (2) for unit dry weight of leaf instead of unit area. Thus the left side of the equation, i. e., lethal deficit in mg. of unit leaf dry weight will be $(D/100)\times 1000$ or $10\,D$ mg. Next if we express the amount of transpiration (T) in mg. for g. dry weight and hr. under the saturation deficit of 10 mm. Hg., $\boldsymbol{T}$ in the right side for one minute will become $T\times(d/10)/60$ or $Td/600$ mg. on the assumption that the transpiration amount has linear relation with the saturation deficit (d) during the dehydration process, which is usually the case in calm air when leaf temperature is nearly equal to air temperature. Then equation (2) will be,

$$10D=\int_o^t \frac{Td}{600}dt \quad \text{or} \quad D=\int_o^t \frac{Td}{6000}dt \text{ (mg.)} \tag{10}$$

Then, T can be expressed in the following empirical formula likewise in the special analysis above mentioned.

$$T=Ae^{-kt}+C \tag{11}$$

Substituting the value of T into equation (10) and after calculation we get,

$$\log_{10}\left\{Ct+\left(\frac{A}{k}-\frac{6000D}{d}\right)\right\}=\log_{10}\frac{A}{k}-0.4343kt. \tag{12}$$

The value of dehydration resistance ($\boldsymbol{t}$) can be obtained by the graphical solution of equation (12), and likewise in the special analysis, when

$$\frac{1}{C}\left(\frac{6000C}{d}-\frac{A}{k}\right) \gtreqless \frac{1}{0.4343k}\log_{10}\frac{A}{k}, \tag{13}$$

then,

$$t=\frac{1}{C}\left(\frac{6000D}{d}-\frac{A}{k}\right). \tag{14}$$

And also in resistant cases,

$$t=\frac{6000D}{Cd}. \tag{15}$$

The discussion of general analysis will be mentioned in the next paper focussing on pine yearlings.

Summary

An attempt was made to express dehydration resistance quantitatively as the time required from detaching to kill the plant part by water loss under a given condition using some mathematical formulae.

1. In leaves whose leaf area can easily be determined, special analysis was put forward by the equation of dehydration resistance (4) and by that of the time trend of relative transpiration (5). Dehydration resistance in minutes (t) was expressed by the following measures concerning the water economy of detached leaves: the tendency of transpiration decrease (k'), the initial relative stomatal transpiration (A'), the final relative transpiration or, in usual cases, relative cuticular transpiration (C'), the lethal dificit (D), the areal weight (M), saturation deficit (d) and the evaporation of leaf-shaped evaporimeter for unit time and area (E). The value of dehydration resistance (t) can be obtained by the graphical solution of equation (6). But when the inequality (8) is fulfilled the solution can be simplified (7) and in resistant cases the time trend of relative transpiration can be disregarded (9). In the case of mulberry plant, simplified solution can only be applicable to normal leaves and the time trend can never be disregarded in all cases.

2. In special analysis the product DM has much bearing on the value of dehydration resistance. Calculating from Pisek and Berger's data[2]), succulent species is the mightiest due to the large value of both D and M, and some conifers are resistent due to large M and a juicy herb, *Veronica baccabunga*, due to large D. Other deciduous trees and herbs are susceptible due to the small value of DM.

3. In general analysis including those plants whose leaf area can hardly be determined, the time trend of transpiration (11) was expressed as mg./g. dry weight/hr./10 mm. Hg. instead of relative transpiration and the similar calculation to the special analysis was conducted, though the analysis could be less thorough, for the measure M could not be included in general analysis.

The author wishes to express his most cordial thanks to Dr. M. Monsi, Prof. of the University of Tokyo, and to Dr. K. Hôgetsu, Prof. of Tokyo Metropolitan University, for their kind advice and criticism throughout his investigation. Thanks are also due to Messrs. T. Ushijima and T. Murakami to their helps for preparing the text.

References

1) Tazaki, T., Bot. Mag. Tokyo **73**: 148 (1960). 2) Pisek, A., and Berger, E., Planta **28**, 124 (1938).

摘　　要

高等植物の乾燥抵抗に関する研究 II. 乾燥抵抗の理論的考察

田　崎　忠　良

一定の条件下で植物体の一部を切りはなしてから乾燥死にいたる時間として，乾燥抵抗を数式で表現した。葉面積が簡単に測定できる葉では，乾燥抵抗の式と摘葉後の比較蒸散量の経過の式によって解析をおこなった．乾燥抵抗は水分経済に関係したつぎの諸量によって表現した．――蒸散減少の傾向 (k')，最初の比較気孔蒸散量 (A')，最後の比較蒸散量すなわち普通は比較クチクラ蒸散量(C')，致死飽差(D)，葉面積重 (M)，飽差 (d) および葉型蒸発計の単位面積・単位時間の蒸発量 (E)――。乾燥抵抗 (t) は (6) 式をグラフによって解くことによって求められる． しかし不等式 (8) が満足されるときは，より簡単な (7) 式により求められ， また抵抗の強い場合には蒸散変化の経過を略することができる(9式)．クワの場合は (7) 式は正常な葉だけに適用され，(9) 式はどの場合にも適用できない．またこの解析によって D とMの積が乾燥抵抗に特に関係があることが明らかにされた．多肉植物は D も M 大きいので抵抗はいちばん強く，針葉樹は Mが大きいためまた多汁の草本は D が大きいため抵抗が大きい．ほかの落葉広葉樹や草本では DM が小さく抵抗が弱いが，D と M の値は種類によって非常にちがう．

葉面積のはかりにくい場合もふくむ場合の解析では，比較蒸散量のかわりに一定飽差下の単位乾量あたりの蒸散量をつかって，前の解析と同じ経過によって解析を行った．しかし解析は特別の解析の場合ほど完全にはゆかない．(東京農工大学繊維学部)

On *Streptomyces aerocolonigenes* nov. sp., Forming the Secondary Colonies on the Aerial Mycelia*,**

by Ryuji SHINOBU*** and Mineko KAWATO***

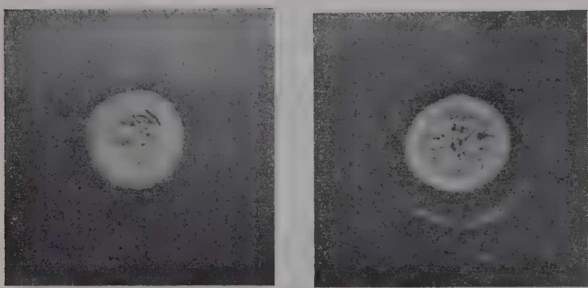
Received October 26, 1959

The strain, No. 701 isolated from the soil at Nagasaki City in October, 1958, formed many little colonies on the aerial mycelia, especially on the cottony ones. The characteristics of this strain were carefully compared with the known species described in Bergey's Manual, 7th Edition[2]), and other reports[3,4,5]) so far published. As the result, this strain was decided to be a new species, and the details of it will be given in the following.

I. Morphological Characteristics

1. Macrocolony

Somewhat unstable; concentric pattern on glycerine starch glutamate agar; a little convex and wrinkled colony at the center. (Photo. 1, A and B)



A

B

3 cm.

Photo. 1: Macrocolony

A and B were cultivated on glycerine starch glutamate agar at 28–30° for 20 days.

2. Microscopical observation

Glucose asparagine agar was mainly used for the microscopical observation, because of the comparatively good growth of the aerial mycelia, and the observation**** was carried on upon the colony cultivated for 3–15 days at 28–30°.

Aerial mycelium: somewhat cottony and wavy on glucose asparagine agar; sometimes a little wetted; monopodial branching; no whirl; no spiral; about 0.8 μ in width.

Many little colonies could secondarily be seen on the aerial mycelia, especially on the cottony ones. As the mycelia of the secondary colonies were short and the spore formation occurred in 4 to 7 days at least, each secondary colony looked like a mass of conidia. (Photo. 2, A, B, C, D, and E)

Conidia: spherical to oval; about 0.8–1 μ in length.

Substrate mycelium: long and wavy; about 0.4–0.6 μ in width.

II. Physiological Characteristics

1. Tyrosinase reaction: positive

* The outline of this study was already reported at the 24th General Meeting of Botanical Society of Japan (1959).

** As for the description of this species, Shinobu's method[1]) was adopted.

*** Hirano Branch, the Osaka University of the Liberal Arts and Education, Osaka, Japan.

**** For the microscopical observation the direct observation method and Shinobu's screen method[6]) were employed.

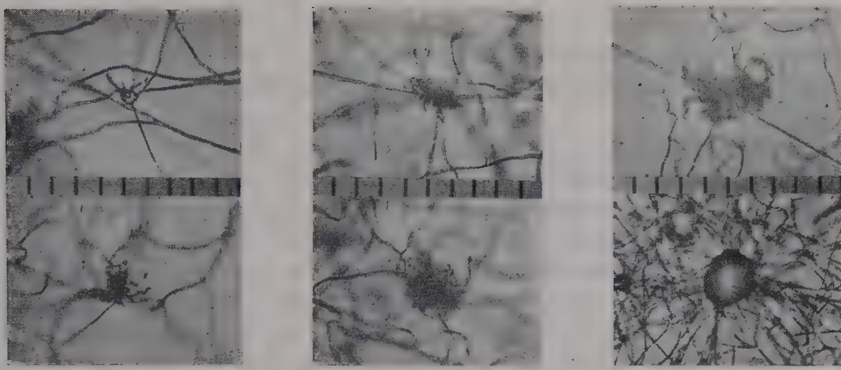


Photo. 2: Formation of the secondary colony
A, young colony; B and C, moderate; D and E, matured ones; F, spherical mass of spores.
All of them were cultivated on glucose asparagine agar at 28–30°. A, B, C, and D for 5 days; E for 7 days; F for 10 days.

2. Nitrite production: positive
3. Diastase reaction (iodine reaction): positive; moderate
   Enzymatic zone (Clear zone):
   - 4–6 mm. on starch agar (Waksman's A) after 8 days' cultivation
   - 6–8 mm. on glycerine starch glutamate agar after 8 days' cultivation
4. Konjakmannase reaction: positive and weak
5. Utilization of carbon sources
   Xylose, fructose, galactose, lactose, trehalose, mannitol, and inositol were utilized.
   Raffinose was not utilized.
   Rhamnose was doubtful.

III. Cultural Characteristics

Notes:
- G ......growth of the colony
- A ......formation of the aerial mycelium and its color
- S ......color of the substrate mycelium
- P ......production of the soluble pigment

1. Ammonium Czapek agar
   G: moderate—poor; thin
   A: none—trace; partial and thin; brownish white
   S: brown—yellowish brown—Golden Yellow
   P: uncertain; probably none
2. Glycerine Czapek agar
   G: good; sometimes net-like growth
   A: trace; small white patches
   S: Tan—Ambergrow—yellowish brown
   P: brown
3. Glucose asparagine agar
   G: good—moderate; somewhat thin
   A: moderate; thin; somewhat cottony; white
   S: Buff

P : pale brown

4. Ca-malate agar
   G : good—moderate; somewhat thin
   A : poor; partial; small white or brownish white patches
   S : brown
   P : pale brown
5. Starch agar (Waksman's A)
   G : good—moderate; somewhat thin
   A : moderate—poor; many small white patches
   S : Apricot Yellow—pale dull yellow orange
   P : none
6. Urea glycerine agar
   G : good—excellent
   A : poor; thin; sometimes partial; pinkish white—brownish white—yellowish white
   S : light brown—Tan
   P : Tan
7. Glycerine starch glutamate agar
   G : good—excellent
   A : moderate—poor—none; thin and partial; white
   S : Buff—Golden Yellow
   P : pale brown

IV. Habitat

Soil (at Nagasaki City)

V. Consideration

No. 701 had a tendency to become weak and to lose its formation ability of the aerial mycelia by the successive cultures, though it formed the white aerial mycelia on various media. Generally, the growth of the aerial mycelia were thin or partial, and the patterns of the macrocolony were concentric at the periphery; and convex and wrinkled at the center, though they were somewhat unstable.

On various media, such as potato peptone glycerine agar, glucose asparagine agar, and glycerine starch glutamate agar, etc., No. 701 did not form any whirl and spiral, but formed many little colonies secondarily on the aerial mycelia, especially on the cottony ones. At first, some short branches came out resembling bushes on certain spots of the aerial mycelia, and later they became a mass of spores on account of sporulation, which occurred after 4 to 7 days' cultivation. (Photo. 2, A, B, C, D, and E) As these secondary colonies were fragile, they were crushed and diffused easily under the weak pressure. (Photo. 3) When a droplet was produced in the neighbourhood of the secondary colony, it turned the colony into a spherical mass, due to the surface tension of droplet, as in the case of *Streptomyces massasporeus*[5]). (Photo. 2, F)

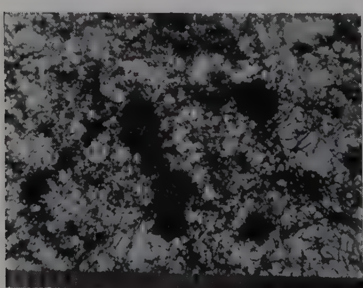


Photo. 3: Crushed colonies under the weak pressure

There could not be seen any remarkable characteristics in the shape and the thickness of the substrate mycelia and conidia.

In order to compare these characteristics of No. 701 with the physiological and cultural characteristics

of the species reported in the past, various experiments, i. e., milky-, gelatin-, cellulase-' invertase-reaction, etc.; and many media, i. e. Bouillon agar, nutrient agar, glucose peptone agar, glucose broth, potato plug, carrot plug, and egg medium, etc. were attempted besides the media stated above. Some important characteristics of No. 701 will be summarized in the following.

No. 701 formed the white aerial mycelia on many media. The substrate mycelia produced brown or brownish pigment, and sometimes yellow or orange series ones. Water soluble pigments (brown—brownish) were often produced on some synthetic media.

Now, there are many species which apparently resemble this strain in some points, especially in the color of the aerial mycelium and in the pigment production. They are *Streptomyces albus*[2,3,4,7] (Rossi-Doria emend. Krainsky) Waksman and Henrici, *Streptomyces longisporus*[2,3,4] (Krassilnikov) Waksman, *Streptomyces albidus*[2,3,4] (Duche) Waksman, *Streptomyces felleus*[2,3,8] Lindenbein, *Streptomyces achromogenes*[2,3,9] Okami and Umezawa, and *Streptomyces globisporus*[2,3,4] (Krassilnikov) Waksman. But none of them formed a secondary colony on the aerial mycelia. Furthermore, the former three formed spiral, while No. 701 did not. The morphological and cultural characteristics of the latter three are different from those of No. 701 as below.

		Str. felleus	*Str. achromogenes*	*Str. globisporus*	*Str.* No. 701
Sporophore				short, often gathered together in thick tufts or bushes	no tufts or no bushes
Spore			cylindrical		spherical～oval
Color of the substrate mycelium	On potato medium	reddish pigment	reddish brown (soluble)		dull yellow
	On egg medium		reddish brown		growth: poor; no reddish brown
Soluble pigment	On synthetic medium			none	brown
	On glucose asparagine agar	none			pale dull yellow orange

Upon the basis of the results stated above, No. 701 was decided to be a new species and was named '*Streptomyces aerocolonigenes*', as the secondary colonies were often formed on the aerial mycelia.

VI. Conclusion and Summary

No. 701 isolated from the soil at Nagasaki City, October, 1958, as one of the *Actinomycetes* strains, formed, secondarily, many little colonies on the aerial mycelia. It was found to be a new species in comparison with those species reported so far in their morphological, physiological and cultural characteristics. The authors will advocate to give it the new specific name '*Streptomyces aerocolonigenes*'.

References

1) Shinobu, R., Memoirs of the Osaka Univ. of the Liberal Arts and Education, B. Natural Science **7**: 1 (1958). 2) Bergey's Manual of Determinative Bacteriology, Baltimore, 7th Ed. (1957). 3) Waksman, S. A., and Lechevalier, H. A., Actinomycetes and their Antibiotics, Baltimore (1953). 4) Krassilnikov, N. A., Guide to the identification of bacteria and Actinomycetes (Edited by Routin, J. B., Pfizer, 1957). 5) Shinobu, R., and Kawato, M., Bot. Mag. Tokyo **72**: 283 (1959). 6) Kawato, M., and Shinobu, R., Memoirs of the Osaka Univ. of the Liberal Arts and Education, B. Natural Science 8: 114 (1959). 7) Waksman, S. A., Soil Sci. 8: 71 (1919). 8) Lindenbein, W., Arch. Mikrobiol. **17**: 361 (1952). 9) Okami, Y., and Umezawa, H., Jap. Jour. of Med. Sci. and Biol. **6**: 266 (1953).

摘　　要

気中菌糸に第二次のコロニーを形成する新種 *Streptomyces aerocolonigenes* について

信夫隆治・川戸峯子

1958年10月，長崎市の土から分離した放線菌の一種 No. 701 は気中菌糸に多くの小型 colony を形成する．本菌を従来記載された species と形態的，生理的および培養的諸性質を比較した結果，新種であることがわかったので *Streptomyces aerocolonigenes* と命名することを提唱する．（大阪学芸大学平野分校）

Diurnal Fluctuation of Chlorophyll Content in Lake Water

by Shun-ei ICHIMURA*

Received November 12, 1959

As bleaching a remarkable decolorization of chlorophyll in the algae which were cultured under a strong illumination has well been known by plant physiologists, while a similar phenomenon has recently been observed in marine phytoplankton under field condition by Yentsch and Ryther[1]), and Shimada[2]). According to their studies, chlorophyll content in the surface phytoplankton exhibits a diurnal fluctuation with its maximum in the early morning and its minimum in the late afternoon. This diurnal fluctuation, if occurs generally and with a considerable amplitude, should be investigated more in detail in order that the chlorophyll method can be employed unanimously in the study of primary production in waters. Hence, the present study has been undertaken to clarify the feature of the diurnal chlorophyll fluctuaion in lakes and its ecological meaning for the primary production.

Methods

For the in situ experiment, the water collected from the center of the surface of a lake was filled in large colorless polyethylene bags of 50 liters. Then these bags were floated on the lake surface where the sampling was made. The necessary aliquot for determination of chlorophyll concentration was taken from each bag at two-hour intervals throughout the day. Simultaneously, sample water was also taken at the depth of 0, 1, 2 meters, etc., in situ near the bags. The sample water of 2 to 3 liters was filtrated through a membrane filter and the latter was treated with steam for about a minute according to Gessner[3]) and dried in the air. Then the membrane filter was put into 10 m*l*. of 80 per cent aqueous weak acid aceton in a 50 m*l*. flask, whereby chlorophyll was extracted as pheophytin. After incubating the flask 24 hours in the dark, the pheophytin was separated from the aceton solution by adding 5 m*l*. benzol and determined in its concentration with the photoelectric colorimeter using a red filter. The chlorophyll concentration was computed by multiplying a conversion factor of 1.026 to the pheophytin concentration determined. As to light condition the daily course of illumination on the lake surface was observed with the photoelectric cell.

Diurnal fluctuation of chlorophyll content in lake waters

Several features of the diurnal fluctuation in chlorophyll content of the surface water in lakes Haruna and Kizaki are illustrated in Fig. 1. From the results it could be surmised that a definite diurnal fluctuation in the chlorophyll content occurred only on clear days, especially during the summer season, but the fluctuation was very slight in winter. The chlorophyll content reached its maximum at around 6 a. m. and thereafter it declined continuously up to the late afternoon. During nighttime,

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.

however, the amount of chlorophyll increased and attained again a high peak in the following morning. The chlorophyll in lakes Haruna and Kizaki showed that the maximum content in the early morning was respectively about 2.3- and 1.5-fold as high as the minimum in the late afternoon. These amplitudes accorded fairly well with those reported by Yentsch and Ryther on the marine phytoplankton in Woods Hole Harbor. The author's further measurements in lakes in Japan have revealed that the diurnal fluctuation in chlorophyll content so far did not exceed 3.0 times even in the surface sample which indicated usually the heaviest fluctuation on clear days. On overcast days, however, the fluctuation was very slight, and moreover the sample in deeper layer indicated no definite diurnal rhythm even under full sunlight. Fig. 2 illustrates the diurnal change in vertical distribution of chlorophyll in lake Haruna on a clear day in June. The fluctuation in chlorophyll content could be observed only in the surface layer and the pattern of productive structure of the phytoplankton community[4]) was slightly changed through the day. Therefore, the diurnal fluctuation in chlorophyll content of the surface layer may not result from the diurnal vertical migration of phytoplankton and the sampling error associated with its heterogeneous distribution. In support of this hypothesis more substantial evidences were obtained with the simultaneous determination of chlorophyll in the samples taken from floating bags and in situ. As seen in Fig. 1, the features of diurnal fluctuation in both cases closely coincided with each other. These results may also illustrate that the diurnal fluctuation in chlorophyll resulted not only from the growth and death of the phytoplankton or cell division in dark period but rather from the oscillation in chlorophyll content within a cell which might be brought about by the diurnal change in illumination.

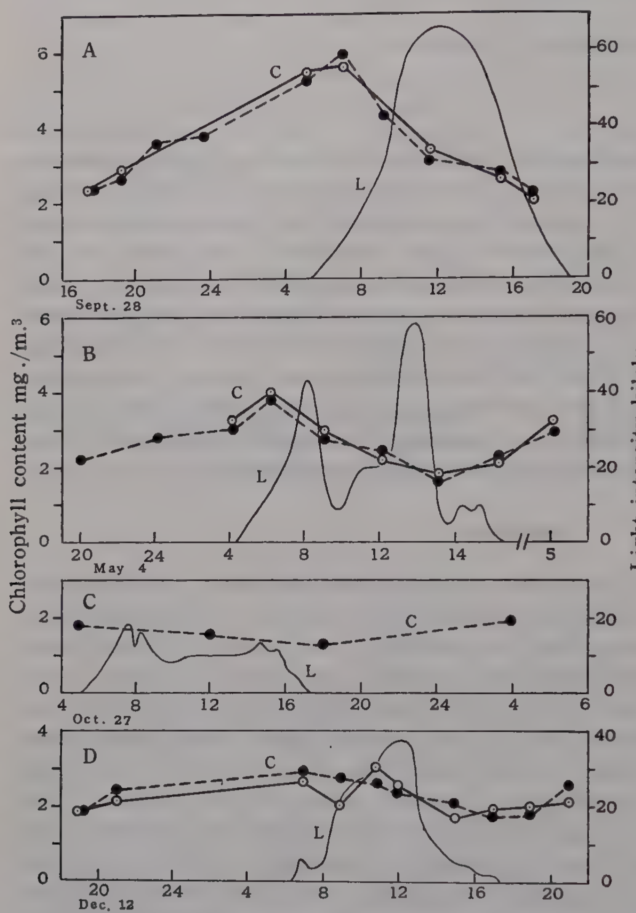


Fig. 1. Diurnal fluctuations of chlorophyll content in surface water of some lakes. A-C, lake Haruna; D, lake Kizaki.

—○—, in situ; - - - ● - - -, in the bag.

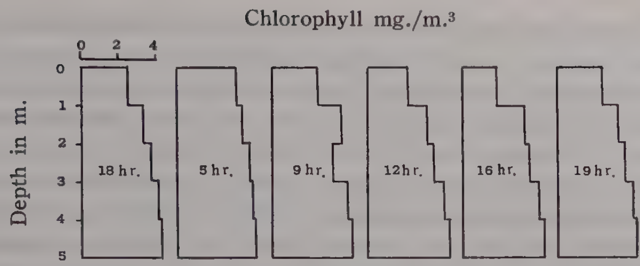


Fig. 2. Diurnal change in vertical distribution of chlorophyll in lake Haruna. Data obtained on June 25, 1958.

Rate of change in chlorophyll content of water and its relation to light intensity

As an example of the rate of change in chlorophyll content, the results obtained in lake Haruna are shown in Fig. 3. The highest rate throughout the day was

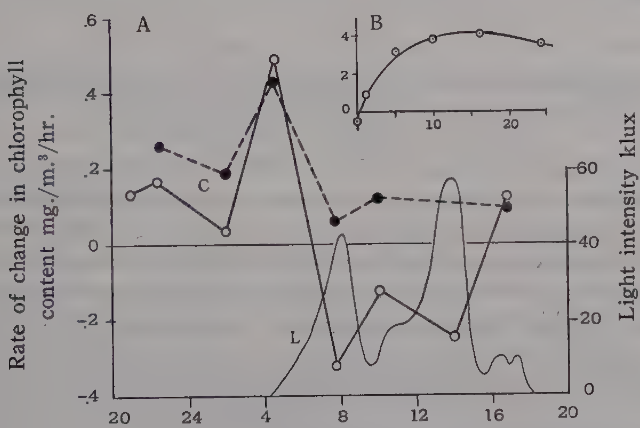


Fig. 3. A. Rate of change in chlorophyll content in surface water and diurnal change in solar illumination in lake Haruna on May 5, 1958.
——○——, in situ; - - - ● - - -, under constant light intensity of 6000 lux.
B. Light-photosynthesis curve obtained in sample water from lake Haruna. Abscisa indicates photosynthetic rate (mg. O_2/mg. chl./hr.) and ordinate is light intensity (klux).

observed in the increase just at daybreak and it was immediately followed by reduction in the rate. In the daytime, especially on clear day, the increase in chlorophyll was rather inhibited and the rate became negative. However, the rate recovered gradually from the negative value to the positive in the late afternoon and continued the positive value in the nighttime.

The rate measured experimentally under a constant light condition differed a little from that observed under field conditions. The sample taken from in situ was exposed for 2 hours to a constant artificial illumination of 6000 lux as performed by Yentsch and Ryther[1]), and then the change in chlorophyll content during the exposure was determined (Fig. 3–A). The results obtained may suggest that one of the

most important factors causing the diurnal fluctuation in chlorophyll content is light intensity. For delineation of the relationship between the change in chlorophyll content and light intensity, the lowest limit of light intensity inhibiting the chlorophyll increase was examined statistically from the data obtained in the field and was generally 20 to 30 kilolux under field conditions. Fig. 3-B indicates the light-photosynthesis curve obtained in the sample water of lake Haruna. In this curve, light saturation of photosynthesis was found at 15–20 kilolux and further increase in light intensity rather reduced the photosynthesis as reported recently by several investigators. Therefore, as suggested by Yentsch and Ryther[1]), it can be inferred that the chlorophyll content increases with light intensity increase in the range where photosynthesis can linearly increase with the latter but it decreases under the too strong light condition which inhibits the photosynthesis. These facts also suggest that there are some interrelationships between photosynthesis and change in chlorophyll content. Incidentally, it is noticeable that a decline of chlorophyll content can be seen just before daybreak, though its real causes are still unknown. The mechanism of the diurnal change in chlorophyll content seems to be of great interest physiologically and biochemically, but the present study will not touch this problem.

Geographical difference in the range of diurnal fluctuation of chlorophyll content in surface waters

Because of insufficiency in data it is very difficult to delineate the character of geographical variation in the range of fluctuation in chlorophyll content. It seems, however, that the range is geographically not so great as that in photosynthetic rate. Since Doty[5]) reported that there were two major variations in photosynthesis with time and latitude of marine phytoplankton even under the same light condition, the periodicity in photosynthetic activity was re-examined in phytoplankton by

Table 1. Geographical variation in the range of diurnal fluctuation of chlorophyll content in surface waters.

Water	Date	Chlorophyll mg./m.^{3}		Ratio	Investigator
		Max.	Min.		
Fukami-ike	Aug. 8, 1959	9.8	4.1	2.5	Ichimura
Lake Haruna	June 8, 1958	3.9	1.7	2.3	〃
Jōnuma	Sept. 12, 1958	174.2	87.4	2.0	〃
Lake Yamanaka	July 21, 1957	1.8	0.9	2.0	〃
Kasumigaura	Aug. 23, 1958	36.5	19.3	1.9	〃
A pond in Osaka	Nov. 11, 1958	26.9	15.0	1.8	Aruga (unpublished)
Lake Kizaki	Dec. 12, 1958	3.0	1.8	1.6	Ichimura
Lake Haruna	Dec. 16, 1958	3.9	2.5	1.5	〃
Woods Hole Harbor	July 18, 1957			2.0	Yentsch & Ryther[1])
Clarion Island	May 28, 1958	0.15	0.08	1.9	Shimada[2])
Sugajima, Ise-Bay	May 17, 1959			1.8	Saijo (unpublished)

several investigators at various latitudes. According to their results, the variation range has been found to be 5-to 6- sometime 10- to 12- fold near the equator but to be usually 1- to 2- fold at high latitude. On the contrary, the diurnal fluctuation in

chlorophyll content shows at every station similar characteristics without latitudinal difference. The range of chlorophyll fluctuation in the surface water measured at several places on clear days is summarized in Table 1, which shows that there occur 1.5- to 2.0-fold variations between the maximum and minimum values. This diversification, though it is rather slight, may be due to the difference in experimental condition such as light intensity, temperature and concentration of nutrients, especially of nitrate. Moreover, it is interesting to note that a 2-fold variation in chlorophyll rhythm was measured at 18 deg. N. latitude[2]), while Doty[5]) found a 5.7-fold variation in the photosynthetic activity near the equator (from 7 deg. N. to 8 deg. S. latitude). Regarding these two periodicities, Yentsch and Ryther have found in both cases 2-fold variations examining marine phytoplankton from about 41 deg. N. latitude.

Ecological meaning of diurnal fluctuation of chlorophyll content on primary production

In the study of phytoplankton production, the amount of chlorophyll in waters has been employed as a basis of routine technique to measure the standing crop and also an index of productive capacity of waters. Moreover, recently Ryther and Yentsch[6]), and Ichimura and Saijo[7]) have carried out the estimation of primary production in the ocean on the basis of chlorophyll amount in waters. By this way, which is known as the chlorophyll method, the primary production in water is calculated; some factors such as vertical distribution of chlorophyll, light condition in water and light-photosynthesis curve being combined on several hypotheses for the photosynthesis of algae. When chlorophyll is adopted as a measure for the standing crop of algae and the sampling is made at various times in a day, it is required to pay attention on the diurnal fluctuation in chlorophyll content for the comparison of the crop in waters. Especially, the correction for such diurnal fluctuation may be most indispensable to the assessment of the standing crop of phytoplankton in a shallow water and of benthic algae in a clear, small stream, where the algae are being exposed to full sunlight.

Since the data on the daily periodicity in the chlorophyll content are very scanty for the present, mathematical formulation for the correction concerning the periodicity may not be easily made, but such seems possible by a graphic method

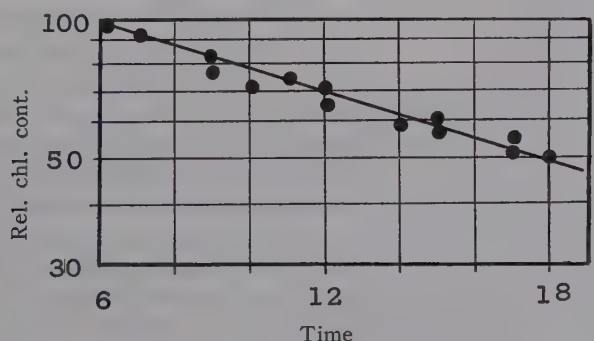


Fig. 4. Range in variation of diurnal fluctuation of chlorophyll content in surface waters.

with making rough estimation of the correction factor. For the purpose of this, Fig. 4 is constructed by compiling the data obtained by Yentsch and Ryther[1]), and

Shimada[2]) as to the ocean and those taken by the author for freshwaters. Each value concerned was determined at the surface layer on a fine day and expressed in a percentage of the maximum value in the morning time. The feature of decreases in chlorophyll content during daytime is roughly expressed by a linear line and a factor of ca. 2 could be applied to the value at 1800 in order to convert it to the value at 0600. Therefore, using Fig. 4, it is possible to approach to the given value at the expected time from the amount of chlorophyll measured at any time on fine days.

The correction for diurnal fluctuation of chlorophyll at various depths may be roughly possible by the following graphic method. The light intensity (I) in water at the depth of t is expressed by a well known equation $I=I_0e^{-at}$, where $I_0$is incident light and a the extinction coefficient. The extinction coefficient is given empirically by $a=1.9/d$ in Japanese lakes, d being the depth of transparency. In case the daily change in light intensity on the surface of water is given, the light intensity at any time at various depths can be estimated indirectly by using both equations. Consequently, the duration times in a day when the plankton is exposed to a light intensity with bleaching effect of above 30 kilolux can be estimated at each depth in a lake. Fig. 5 indicates the relationship between the depth in lakes with various transparencies and the duration times exposed under the light of above 30 kilolux in the summer season.

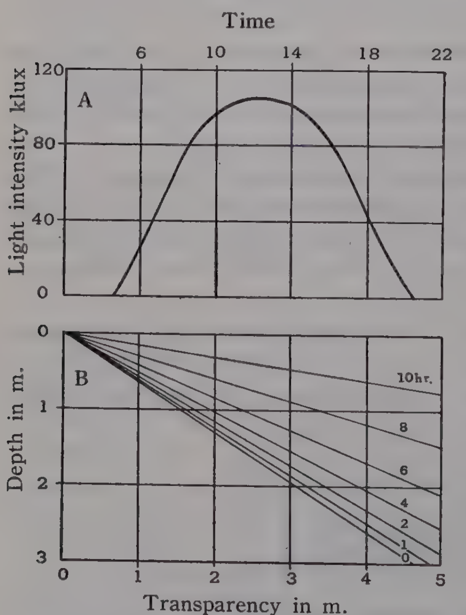


Fig. 5. A. Diurnal change of illumination in July in Tokyo.

B. Relation between depths in lakes with various transparencies and duration times exposed under light of above 30 kilolux under light condition of A.

As seen in Fig. 4, on the other hand, the chlorophyll seems to decrease as a linear function of the duration time regardless of light intensity in the range of above 30 kilolux. After a 12-hour duration in situ on a fine day, the chlorophyll content declines to a minimum, being reduced to about a half of the maximum in the morning time. Considering the above hypothesis of the light effect on the chlorophyll bleaching, the phytoplankton which has been exposed 6, 3 and 1 hour to light intensity of above 30 kilolux decrease their chlorophyll amout to 3/4, 7/8 and 23/24, respectively. Therefore, the correction factors for each depth can be obtained graphically by the same method employed in Fig. 4. In a eutrophic lake with transparency depth of 1 meter, the duration time of the inhibitory strong light intensity is only one hour at the depth of 60 cm. In the surface layer of a lake, on the other hand, the photosynthetic activity of phytoplankton is usually reduced by strong illumination and the standing crop is also poor, thereby the proportion of the organic matter produced in the surface layer is very low in comparison to the total organic matter produced in the phytoplankton community as a whole. For this reason, in determining productivity of phytoplankton community by the chlorophyll method, the error

caused by the diurnal fluctuation in chlorophyll content can be rather small at least in eutrophic lakes.

As a reference, the daily primary production in lake Haruna calculated by the chlorophyll method will be illustrated in the following. This calculation was carried out by using the chlorophyll data measured in June, 1958 (see Fig. 2) and the light-photosynthesis curve with 4.0 mg. O_2/mg. chl./hr. at the light saturation point and 15°. At that time, the depth of transparency was 1.8 meters. The amount of chlorophyll in the surface layer used for calculation of daily productivity was estimated in four ways without correction as to the diurnal fluctuation; in the first was used the chlorophyll content measured at regular interval of 2 hours throughout the day, in the second the maximum chlorophyll content in the morning, in the third the minimum value in the late afternoon, and in the fourth the chlorophyll content at noon. The final results of the calculations, primary productions per lake surface area, were 0.263, 0.295, 0.245 and 0.274 g. O_2/m^2./day, respectively, i. e., relatively, 100: 112: 93: 104. The data presented here may provide a source of evidence that the diurnal fluctuation in chlorophyll content, which is of interest in itself and has large meaning in assessment of standing crop, hardly has a noticeable significance for the chlorophyll method, especially in eutrophic lakes

The author wishes to express his thanks to Prof. M. Monsi and Prof. K. Hogetsu under whose guidance this research has been carried out. Also his thanks should expressed to Dr. Y. Saijo for his kind support in the research.

Summary

The diurnal fluctuations of the chlorophyll content in lake water were measured under field condition in some Japanese lakes.

1. Definite fluctuations are observed in the surface water on a clear day and are very slight in the subsurface layers or on an overcast day. In the daily rhythm, the highest chlorophyll concentration is usually obtained in the early morning and the lowest in the late afternoon, the amplitude being a 2-fold range in the surface water. This fluctuation seems to be caused by the diurnal change in illumination: chlorophyll bleaching occurs in an illumination about 30 kilolux or more.

2. The correction for such fluctuation is required for the surface layer algae of lakes and the benthic ones in small, clear streams, when the chlorophyll content is used in a technique to measure the standing crop of phytoplankton. A graphical solution was submitted.

3. In determining productivity of phytoplankton community by using the chlorophyll method, the error caused by the diurnal fluctuation in chlorophyll is rather small especially in eutrophic lakes, because the surface layer where appears the fluctuation is poor in phytoplankton composed with the subsurface layer in such lakes. Therefore, it is inferred that in many cases the diurnal fluctuation does not appear as a shortcoming for the chlorophyll method.

References

1) Yentsch, C. S., and Ryther, J. H., Limnology and Oceanography **2**: 140 (1957). 2) Shimada, B. M., ibid. **3**: 336 (1958). 3) Gessner, F., Arch. f. Hydrobiologie **40**: 686 (1944). 4) Ichimura, S., Bot. Mag. Tokyo **69**: 7 (1956). 5) Doty, M., Current status of carbon-fourteen method of

assaying productivity of the ocean. Contract AT (04–3)-15, (1958). 6) Ryther, J. H., and Yentsch, C. S., Limnology and Oceanography **3**: 327 (1958). 7) Ichimura, S., and Saijo, Y., Bot. Mag. Tokyo **72**: 193 (1959).

摘　　要

湖沼内のクロロフィル含量の日変化

市 村 俊 英

晴天における湖沼内のクロロフィル含量は，表層水中で，特に日周期的な変動が見られる．一般に最大値は 6 時，最小値はほぼ 17 時に測定され，両者の比は夏季約 2：1，冬季はきわめて小さい．日変化は曇天ではおこらず，また晴天でも深層中ではほとんど見られない．クロロフィルの減少は光合成を阻害するような強光下においてのみ認められる．日変化の現象は植物プランクトンの垂直移動によるものではない．光および光合成と密接な関係があり，生体内のクロロフィル自体の変化とも考えられるがまだ明らかでない．クロロフィル量および光一光合成曲線を用いての基礎生産の解析では，この日変化は富栄養湖では無視できるが貧栄養湖では考慮しなければならない．(東京教育大学理学部植物学教室)

Studies in *Treubia nana* (Hepaticae) with Special Reference to the Antheridial Development

by Hiroshi INOUE*

Received November 25, 1959

*Treubia*** is a peculiar genus occupying the intermediate position between the thallose and the foliose liverworts. On *T. insignis*, the type of the genus and well known by its largest size among the liverworts, many papers have been published on its anatomical nature. The second species, *T. nana*, treated in this paper, has been poorly known by the original description of Hattori and Inoue[1]) and an additional paper of oil-bodies[2]). Their papers were preliminary ones because, at that time, the plants were known only in sterile condition. I have collected this species several times at the type locality, but the sexual organs and sporophytes were not found. It has been known nowhere else.

On July 20, 1959, I could collect this species on the Kokushi peak of Chichibu Mountains, situated about 13 km. west of Karisaka pass (type loc.). The plants of Kokushi had some young sporophytes and near shoot apex many antheridia. The structure and the development of the antheridia of *Treubia insignis* have not been observed in detail, except for short descriptions by Stephani[4]) and Schiffner[5]).

The material used for the microtome sections is Kokushi collection. It was fixed with the strong chrome-acetic mixture for 24 hrs. Microtome sections were cut at the thickness of 7 μ and stained in Heidenhain's iron-alum haematoxylin.

Observations

1) *The oil-cell and the oil-bodies*: The oil-bodies were described originally from the dried plant[1]). Consequently Inoue and Hattori[2]) described them from living plants, and said that "The oil-body type of this species and *T. insignis* is just the same, and differs from that of all other liverworts." This conclusion is verified also in the present investigation. The oil-cells, containing a single, large, dark brown oil-body and several of chloroplasts (Fig. 2, d, f), are scattered in all the tissue of plant except the central tissue of stem. The oil-cells are 30 40 $\times$ 40-53 μ in leaf-middle and little smaller than ordinary cells which are 30-40 $\times$ 43 57 μ. Chloroplasts are 2-3 μ in each oil-cell. They are little smaller in size and fewer in number than those in the ordinary cells of leaf.

2) *The structure of stem and leaves*: Plants are dark green and about 2 cm. long. Stem is prostrate, simple or rarely dichotomously branched. It is flattened dorsiventrally (Fig. 1, c) and, when well developed, about 30 cells thick in the middle portion. In the cross-section, the central tissue is well differentiated and nearly triangular in outline; it is about 10 cells high. The cells of central tissue are parenchymatous (Fig. 2, e) and a little smaller than those in other tissue of the stem. They contain

* Botanical Institute, Faculty of Science, Tokyo University of Education, Koishikawa, Tokyo, Japan.

** *Treubia* comprises three species; *T. insignis* Goeb. in the tropical Asia, *T. nana* Hatt. et Inoue in Japan, and *T. lacunosa* (Colenso) Prosk. of New Zealand. Proskauer[3]) thought that *T. bracteata* St. in Samoa was probably a synonym of *T. lacunosa*.

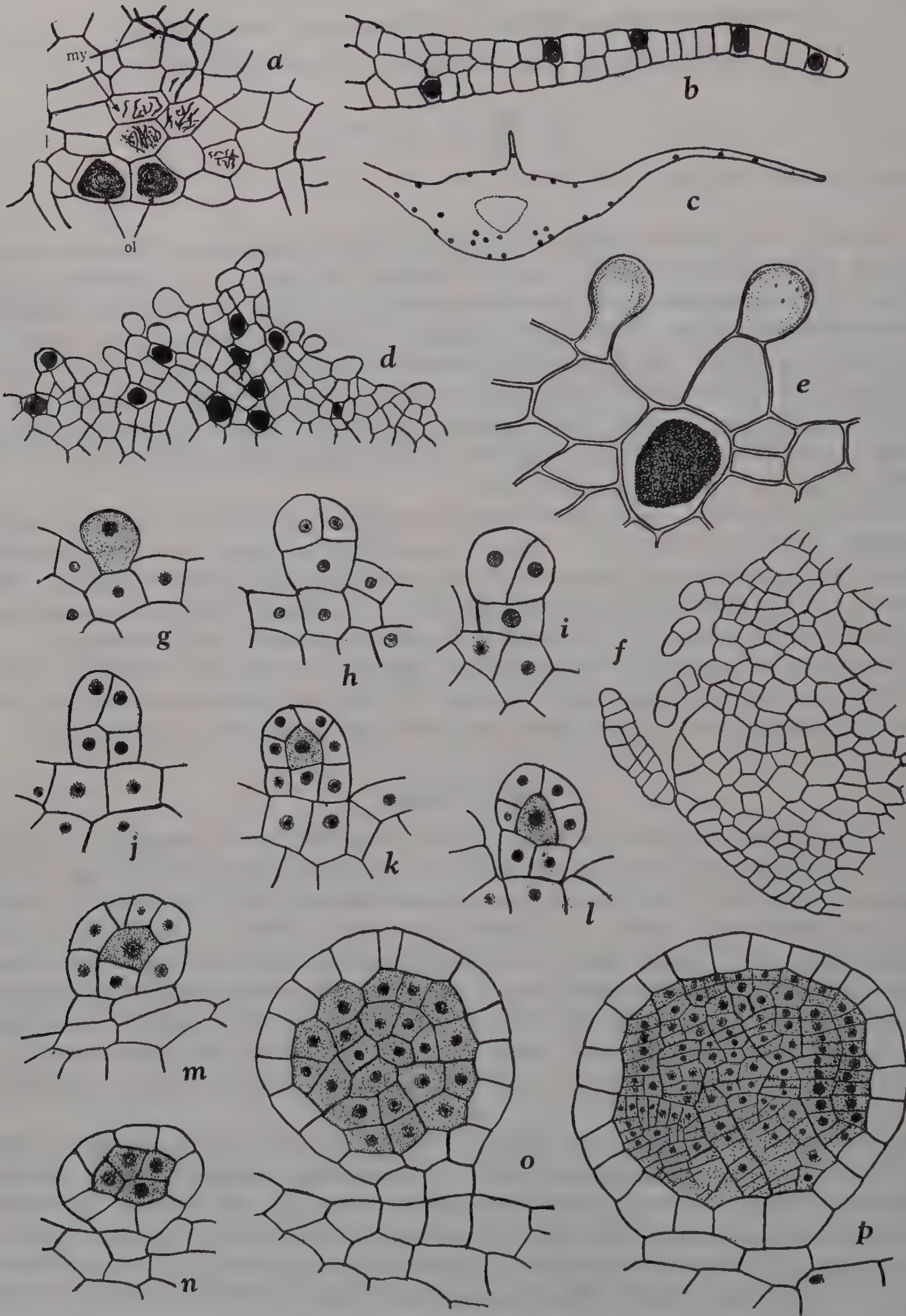


Fig. 1. a. Ventral part of cross-section of stem (my: mycorrhiza. ol: oil-body), ×160. b. Cross-section of leaf, ×160. c. Cross-section of stem (dots indicate the distribution of oil-bodies), ×12. d. Ventral margin of young leaf, ×95. e. Part of (d), showing marginal papillae and oil-body, ×360. f. Longitudinal section of stem apex, ×160, g–p. Various stages of antheridial development, ×360,

numerous oil-drops and diminutive chloroplasts (so that only the central tissue is peculiarly greenish in the inner stem tissue). The other portion of stem is also composed of parenchymatous cells and the cells contain some oil-drops which are colorless, but no chloroplast. The epidermal cells are usually a little smaller than inner cells, and they have no cuticular thickening. In *T. insignis*, Grün[6]) described the cuticle to be thickened and central cells as sclerenchymatous. The cauline tissue passes into the dorsal and lateral leaves (Fig. 1, b). The lateral leaves are about 8 cells thick and the dorsal ones about 3 cells thick at base. Both become gradually one cell thick towards the margine (Fig. 1, b). The oil-cells seem to be more in number in the ventral portion than the dorsal one of the stem, and they lack in the central tissue. Mycorrhizal cells (Fig. 1, a) are often observed in the ventral portion. The rhizoids are colorless and long, and originate from the epidermal cells along the middle of the ventral portion of stem. No hypha is observed in the rhizoidal cells.

The growth of the stem takes place by means of a single apical cell (Fig. 1, f), which has three cutting faces as that of *T. insignis*[6,7]). The cells formed by cutting of the ventral face are smaller than the others. Two cells adjacent to the apical cell in longitudinal section are peculiarly large. The cells at ventral sector do not produce any ventral organ, but the cells derived from the lateral two produce lateral leaves from which the dorsal leaves are developed.

Along the ventral margin of a young leaf (developed near shoot apex) usually are observed some mucilage cells (Fig. 1, d, e). They are always unicellular. Sometimes they are also observed near shoot apex dorsally but very few.

3) *The development of antheridia*: The antheridial development begins with enlargement of an epidermal cell, which is usually 4-5 cells apart from the apical cell. The antheridial initial projects on the epidermis and divides into two cells equal in size, a basal and an outer cell. The basal cell is occasionally embeded in the epidermal cells of stem. The outer cell is primary antheridial cell and the basal one is primary stalk cell. The second cell division takes place in the primary antheridial cell vertically, and the third one in the primary stalk cell also vertically; the cell divisions in the outer and basal cells are not simultaneous. The basal two cells do not make further division and become the antheridial stalk. This stalk, therefore, has height of one cell and width of two cells. The primary antheridial cell makes further division forming sterile jacket cells surrounding a central fertile cell. These jacket initial cells divide to form the one-layered jacket of the antheridium. The fertile cell is divided by tangential and vertical walls into four cells, which repeatedly divide into the androgonial cells.

Thus formed antheridia are globose, measuring about 140 μ in maximum. They are found abundantly at random on the dorsal part adjacent to the shoot apex, without being limited to the keel of the dorsal leaves (Fig. 2 b, j). Paraphysis or similar organs are not present. It is noteworthy that the plants having calyptrae also have many antheridia; thus, *T. nana* is decidedly monoecious species contrary to the dioecious *T. insignis*.

4) *Calyptra and young sporophyte*: The plants used for this study have had several calyptrae, in which there are already developed young sporophytes and is observed no archegonium. The calyptra arises singly a little apart from the axil of the dorsal leaf and is well developed. The calyptral wall (Fig. 2, g, i) is about 7 cells thick at middle to apex and more than 10 cells thick at base. The calyptra is cylindrical and has some few-celled projections, but any unfertilized archegonium is not seen. Campbell[7]) observed unfertilized archegonia on the calyptra of *T. insig-*

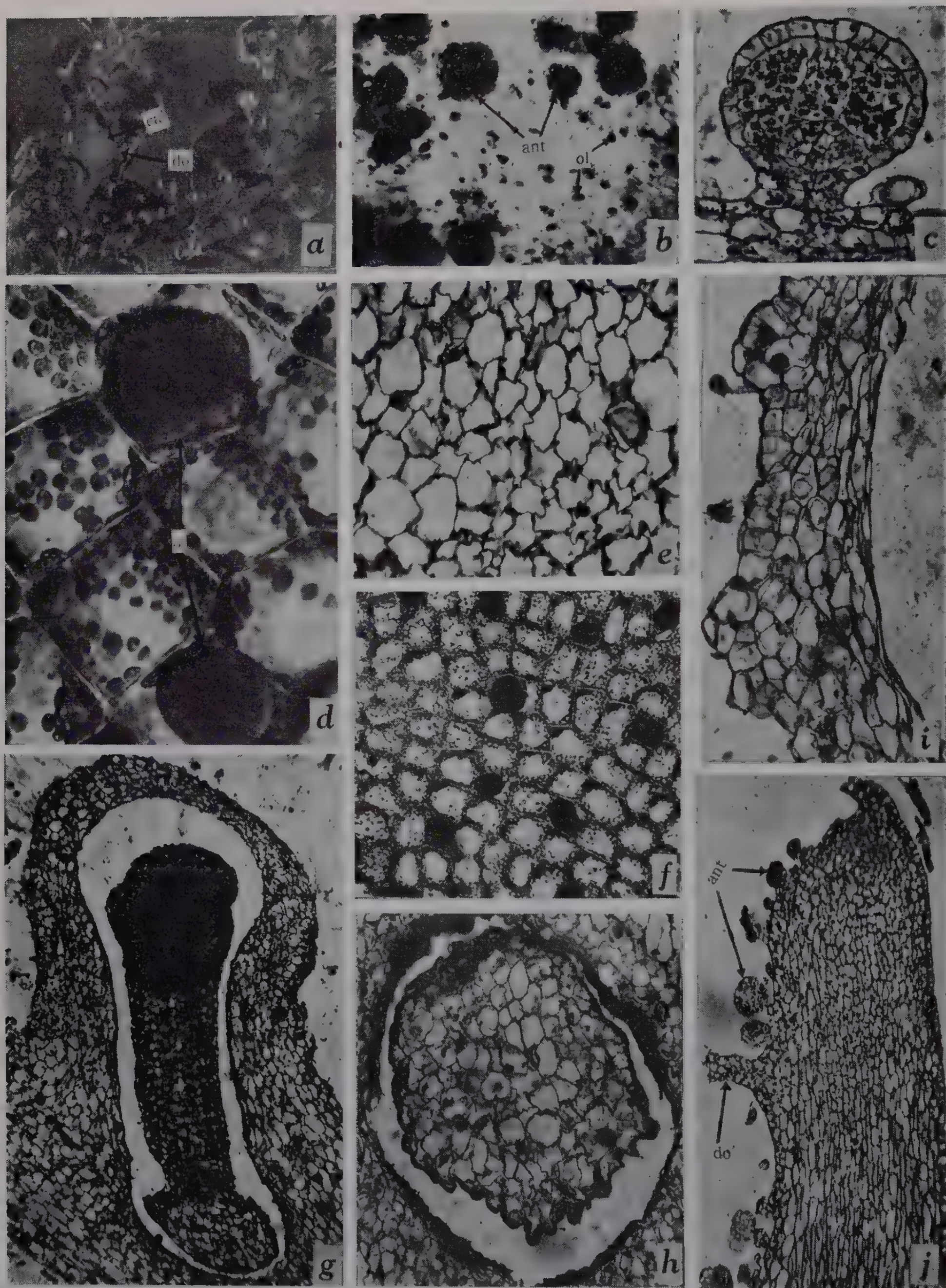


Fig. 2. a. Part of plant, dorsal view (cal: young calyptra. do: dorsal leaf), × ca. 4. b. Dorsal view near stem apex (ant: antheridium. ol: oil-body), ×40. c. Longitudinal section of antheridium, ×140. d. Oil-bodies of leaf, ×800. e. Cells from central portion ot stem, ×400. f. Cells from leaf. middle, ×110. g. Longitudinal section of young calyptra and sporophyte, ×45. h. Cross-section of young seta, ×75. i. Part of longitudinal section of young calyptra, ×65. j. Longitudinal section of stem apex, ×55. All of figs. 1~2 were based on plants collected on Mt. Kokushi, Nagano Pref.

nis. The innermost layer of calyptral wall is the cells of more or less elongated. The oil-cells are often observed in the calyptral wall. In cross-section, the seta of the young sporophyte (Fig. 2, h) is about 12 cells across and has about 40 epidermal cells. The foot is well developed (Fig. 2, g).

Discussions

There are observed many differences between *T. insignis* and *T. nana*. The distribution of the antheridia and their stalk cells seem to be the most remarkable. In *T. insignis*, Stephani[4]) described antheridia, with a figure, as "antheridia usque ad 20, longissime pedicellata." Schiffner[5]) also noted them as the same with Stephani's description (but, his note seems to be based on Stephani's description). Their observations were not verified by the later investigators such as Grün[6]) and Campbell[7]).

The developmental pattern of the antheridia is much different from that of related genera (for example, *Fossombronia* as well known in the literature). Chalaud[8]), Haupt[9,10]) and others studied the development of antheridia in *Fossombronia* species. According to their papers, the antheridial initial of *Fossombronia* divides into two cells, as in *T. nana*, but the outer cell divides further into two cells, an outer antheridial cell and a median stalk cell; the basal cell is always embeded in the stem tissue. In *T. nana* the antheridial initial divides only once and forms a primary antheridial cell and a primary stalk cell. The stalk cell does not divide tangentially, remaining only one cell high. The similar antheridial stalk is observed in S. American *Noteroclada*. Spruce[11]), Leitgeb[12]) and others described the antheridia of this genus, and Schiffner[13]) fully discussed their descriptions and said: "die Verbindungszelle ist von den übrigen Zellen so wenig verschieden, dass sie nicht als Stiel gedeutet werden kann."

According to Stephani[4]), the antheridia of *T. insignis* develop in group along the keel of the dorsal leaves and have long stalks. In *T. nana* they occur rather scatteredly near shoot apex dorsally as in *Noteroclada*. Schiffner's idea[13]) on the relation of *Treubia* to *Noteroclada* is also to be admitted by such antheridial features. It is, however, much desirable to study carefully the antheridia of *T. insignis* to verify the descriptions of Stephani[4]) and Schiffner[5]).

Campbell[7]) suspected the relationship of *Treubia* with *Fossombronia*. In view of the antheridial characteristics of *T. nana*, his suspection seems to be problematic. To settle this it is also desirable to study the antheridial development of *Noteroclada*.

Göbell[14]), Grün[6]) and Campbell[7]) observed in *T. insignis* 3–4-celled characteristic gemmae near shoot apex. These gemmae were not found in *T. nana*. The nature of epidermal and central cells of stem is also different between these two species. Grün[6]) described for *T. insignis* the thickened cuticle and sclerenchymatous central tissue of stem. Furthermore, in *T. nana* there is no scale-like out-growth of calyptra observed in *T. insignis*[6,7]).

Summary

In this paper *Treubia nana* Hatt. et Inoue, Japanese representative of the genus, was studied by microtome section. The antheridial development was described, which has been unknown in other members of the genus. There were found some essential differences between *T. nana* and well-known *T. insignis*. The relationships between *Treubia* and its related genera as *Fossombronia* and *Noteroclada* were discussed from the view point of the antheridial development.

I am much indebted to Dr. S. Hattori and Prof. H. Ito for their much valuable criticisms for this paper.

References

1) Hattori, S., and Inoue, H., Journ. Hattori Bot. Lab. **11**: 99 (1954). 2) Inoue, H., and Hattori, S., ibid. **12**: 116 (1954). 3) Proskauer, J., The Bryologist **58**: 192 (1955). 4) Stephani, F., Hedwigia **30**: 190 (1891). 5) Schiffner, V., Hepaticae in Flora von Buitenzorg, 220 pp., Leiden (1900). 6) Grün, S., Flora **106**: 331 (1914). 7) Campbell, D. H., Amer. Journ, Bot. **6**: 261 (1916). 8) Chalaud, G., Rév. Gen. Bot. 41. (1929-1931). 9) Haupt, A. W., Bot. Gaz. **69**: 318 (1920). 10) ——— , ibid. **88**: 103 (1929). 11) Spruce, R., Trans. Proc. Bot. Soc. Edinburgh **15**: 1 (1884-5). 12) Leitgeb, H., Untersuchung über die Lebermoose III. 144 pp. Jena (1877). 13) Schiffner, V., Östr. Bot. Zeitschr. **61**: 325 (1911). 14) Göbell, K., Ann. Jard. Bot. Buitenzorg **9**: 1 (1891).

Addenda

1. After the manuscript of this paper was submitted to the editor, I received from Mr. N. Kitagawa a reprint of paper, "Occurrence of *Treubia nana* on Mt. Hayachine" in Acta Phytotax. Geobot. **18**: 38, 1959. In this paper Mt. Hayachine was reported as the second known station of this species.

2. In Journ. Hattori Bot. Lab. **21**: 231 (1959) I proposed a new genus of the Calypogeiaceae *Metacalypogeia*. This was the genus elevated from the subgenus of *Calypogeia* which was first described and recognized by Dr. Hattori. At this treatment I cited *Metacalypogeia cordifolia* (Steph.) Inoue as the type species of new genus. This citation was inadequate in the spirit of the International Code of Nomenclature and should be corrected as follows:

Metacalypogeia (Hatt.) Inoue, Journ. Hattori Bot. Lab. **21**: 231 (1959). Type species: *Calypogeia sendaica* Steph. = *Metacalypogeia cordifolia* (Steph.) Inoue.

For the synonyms of the type species see p. 233 of Journ. Hattori Bot. Lab. 2 . I have to acknowledge to Dr. H. A. Miller of Miami University for his kind suggestion.

摘　　要

ヒメトロイブゴケ，特に造精器の発生について

井 上　浩

ヒメトロイブゴケは埼玉県秩父の雁坂峠産のものによって記載されたが，それ以後今日まで生殖器官および胞子体未知のままであった．産地も上記以外には報告がなかった．最近，本種を長野県国師岳において採集したが，国師岳産のものにおいて多数の造精器および若い胞子体を観察することができたので，ここで主として造精器の発生過程とともに二三の知見を加えておいた．

トロイブゴケ属において造精器は Stephani によって記載された以外に Schiffner のかんたんな記載があるのみでその発生過程は明らかではない．ヒメトロイブゴケにおいてみると，造精器の分布はトロイブゴケとは全く異なり南米の *Noteroclada* に類似している．造精器の発生過程も ***Fossombronia*** などの近似の属においてみられるものとは相当異なった過程を示している．このような造精器の形質ならびにその発生過程から考えると，トロイブゴケ属は Campbell の考えと違って，***Fossombronia*** の一群よりもむしろ *Noteroclada* などに近似した形質が多いと考えられる．

またヒメトロイブゴケと *T. insignis* の関係についても基本的なちがいが多く，これらを同種またはごく近似の種とみなすことはできないのではないかと考えられる．（東京教育大学理学部植物学教室）

Anthocyanin of the Seedlings of a *Polygonum*. Studies on Anthocyanins, XXXII*

by Nobuhiko SUGANO** and Kôzô HAYASHI**

Received December 11, 1959

The seedling of a cultivated variety of *Polygonum hydropiper* L. is remarkable in that a pronounced deep red color appears immediately after germination in the cotyledons as well as in the hypocotyl. The seedling is known as 'Benitade', i. e., red knot-grass, and is occasionally used as a garnish in Japanese dishes.

The chemical nature of this pigment has been studied as a preliminary measure for investigating the biochemical process of pigment formation in these seedlings. The pigment was finally obtained as brownish red needles which showed all the characteristics of idaein (3-monogalactoside of cyanidin). Idaein, however, is hardly distinguishable from chrysanthemin (3-monoglucoside of cyanidin) on the paper chromatogram on account of the similarity in the R_f values of both pigments with various solvent mixtures. It was found that the differentiation was achieved by means of paper electrophoresis with dilute aqueous borax solution.

Idaein, sofar, has been isolated from the fruits of the following plants: *Vaccinum vitis-idaea* L.[1]), *Fatsia japonica* Decne. et Planch.[2]), and *Pirus malus* L.[3]). The present isolation is probably the first instance that the pigment has been obtained from vegetative organs.

Experimental

Fresh seedlings (900 g.) were extracted with cold 1% methanolic hydrochloric acid (2 *l*.) for an hour followed by filtration, and the material was again extracted with the same solvent (0.5 *l*.). The combined extract was carefully added with aqueous basic lead acetate, until the precipitation of the blue lead salt was complete. The precipitate was quickly filtered, washed with methanol, and dried. The conversion of the lead salt into chloride was effected as usual by treatment with 4% methanolic hydrochloric acid (200 m*l*.), and the product was precipitated with a large excess of ether (about 6 vols.). The amorphous red powder obtained was dissolved in hot water (60 m*l*.) and filtered solution was mixed with a saturated aqueous solution of picric acid. On cooling, needle-shaped crystals commenced to separate. After standing overnight, the crude picrate was collected and recrystallized from water (80 m*l*.) containing some picric acid. The crystals (0.48 g.) consisted of homogeneous brownish red needles, which were sparingly soluble in cold water, but very easily soluble in hot water, methanol and ethanol. Mp. 162–163° (decomp.).

Conversion into Anthocyanin Chloride.

The purified picrate was dissolved in a small amount of hot water and added with 3.5 vols. of 4% methanolic hydrochloric acid. On standing in a refrigerator overnight, straight-cut needles crystallized out. Recrystallization was repeated twice

* Part XXXI: Proc. Japan Acad., **35**: 169 (1959); Bot. Mag. Tokyo, **72**: 325 (1959).

** Botanical Institute, Faculty of Science, Tokyo University of Education, Ohtsuka, Tokyo, Japan.

in a similar manner. Yield 0.082 g., viz., 0.009% of fresh plant material. The crystalline chloride is moderately soluble in cold water with brownish red color. The color of ethanolic solution is pure red. An aqueous solution of the chloride gives with aqueous ferric chloride a violet color, which turns into intense blue on dilution with ethanol. The distribution number between *iso*-amyl alcohol and 0.5% hydrochloric acid was determined as 10–13, which lies within a range found with a majority of monoglycosidic anthocyanins*. The air-dried specimen melts at 209–210° under simultaneous effervescence. It contains one molecule of water of crystallization. (Found: H_2O 3.61%. Calc. for $C_{21}H_{21}O_{11}Cl \cdot H_2O$: H_2O 3.58%.) CH-Determination of the air-dried specimen gives a result that agrees with the calculated value of monohydrate of cyanidin-monohexoside. (Found: C 50.47, H 4.20%. Calc. for $C_{21}H_{21}O_{11}Cl \cdot H_2O$: C 50.15, H 4.57%.)

Spectrophotometric examination: The crystalline chloride was dissolved in 60% ethanol containing 0.1% hydrochloric acid in a concentration of 5×10^{-5} mol. and the solution was subjected to spectroscopic analysis, chrysanthemin and idaein being examined in parallel. The absorptions of three anthocyanins proved to be quite identical in the entire spectral region, indicating that they were quite similar as to the glycoside bonding.

Paper chromatographic test: Developed with three different solvent mixtures by ascending method, it was found that the R_f values were not sufficient for the discrimination between chrysanthemin and idaein, as shown in the following data:

	Chrysanthemin	Idaein	*Polygonum*-anthocyanin
n-BuOH/AcOH/H_2O [4 : 1 : 5 (v/v)]	0.31	0.27	0.27
Phenol saturated with water	0.26	0.30	0.30
AcOH/conc. HCl/H_2O [5 : 1 : 5 (v/v)]	0.60	0.60	0.60

Paper electrophoresis: A satisfactory method of discrimination between chrysanthemin and idaein was found; that is, paper electrophoresis method in 1% borax solution (pH 11). The following electrophoresis (16 mA/cm., 650 V, Tôyô No. 52 filter paper, one-hour run) clearly demonstrated the identity of the pigment from *Polygonum*-seedling with idaein.

	Chrysanthemin	Idaein	*Polygonum*-anthocyanin
Distance of migration toward the anode	0.21 mm.	0.26 mm.	0.26 mm.

Hydrolysis of the Glycoside.

Identification of cyanidin: When the glycoside was boiled with 20% hydrochloric acid, it was easily hydrolyzed and the aglycone began to crystallize out in brownish red needles while hot; the separation was complete on cooling. The crystals were collected by filtration and air-dried. 27.77 mg. of the aglycone were obtained from 39.65 mg. anhydrous glycoside chloride. (Found: aglycone 70.0%. Calc. for $C_{15}H_{11}O_6Cl.H_2O$ from $C_{21}H_{21}O_{11}Cl$: 70.3%.) The identity of this substance with cyanidin was established by careful comparison with the authentic specimen. An air-dried specimen gave analytical values corresponding to those of cyanidin chloride. (Found: C 52.89,

* Distribution number of monoglycosides: 10 (Willstätter and Mallison), 14.9 (Hayashi) for idaein, and 19.5 (Willstätter and Bolton), 18 (Hayashi) for crysanthemin, respectively.

H 3.86%. Calc. for $C_{15}H_{11}O_6Cl \cdot H_2O$: C 52.88, H 3.58%.) Paper chromatographic data proved also the identity of this substance with cyanidin: R_f 0.31 for cyanidin, and 0.31 for *Polygonum*-anthocyanidin [Acetic acid/conc. HCl/water, 5 : 1 : 5 (v/v)].

Determination of the sugar component: The almost colorless, acidic filtrate obtained above was evaporated to dryness *in vacuo* over solid sodium hydroxide. The residue was dissolved in a minimal amount of water and analyzed by descending paper chromatography on the one hand and by paper electrophoresis on the other. By these methods, the sugar component was determined unequivocally as galactose, as seen from the following data:

Paper chromatography of sugar component (descending, Tôyô No. 52 filter paper, 15°)

	Sugar from *Polygonum*-anthocyanin	Galactose	Glucose
Pyridin/*n*-BuOH/H_2O* (4 : 6 : 3 (v/v))	172 mm.	172 mm.	192 mm.
n-BuOH/AcOH/H_2O** (4 : 1 : 5 (v/v))	56 mm.	56 mm.	70 mm.

* Distance of migration from the start after 40 hrs.
** Do. after 20 hrs.

Paper electrophoresis of sugar component (1.8 mA/cm., 500 V, Tôyô No. 52 filter paper, 3-hour run in 1% borax sol.)

	Sugar from *Polygonum*-anthocyanin	Galactose	Glucose
Distance of electrophoretic migration toward the anode	47 mm.	47 mm.	60 mm.

The authors' thanks are due to Dr. Kazuo Furusato of the National Institute of Genetics (Misima), and also to Messrs. R. Yamaguchi and T. Nukaya for their collaboration in obtaining plant material.

References

1) Willstätter, R., and Mallison, H., Liebigs Ann. Chem., **408**: 15 (1915). 2) Sando, C. E., Journ. Biol. Chem., **117**: 45 (1937); Duncan, I. J., and Dustman, R. B., Journ. Amer. Chem. Soc., **58**: 1511 (1936). 3) Hayashi, K., Acta Phytochim., **11**: 91 (1939).

摘　　要

ベニタデ (芽生え) のアントシアニン (アントシアニンの研究. 第 32 報)

菅 野 延 彦・林 　孝 三

ヤナギタデ *Polygonum hydropiper* L. の栽培品で芽生えが特に赤く色づくものをベニタデと呼んで，古くから料理の「つま」に用いている．この芽生えにおける色素の生成過程をしらべるための前提として，われわれは色素の単離同定を試みた．

ベニタデの赤色はペーパークロマトグラフ的に単一のアントシアンによるものであり，さらに結晶色素の化学分析によりイデイン（シアニジン-3-ガラクトサイド）と同定された．

一般に葉などの栄養器官に出現するアントシアンはクリサンテミン（シアニジン-3-グルコサイド）であり，今回のようにガラクトース配糖体である例は，今までのところ *Fagus sylvatica* の紅葉[Robinson, R., and Smith, H., Nature **175**: 634 (1955)] 以外には知られていない．

なおイデインとクリサンテミンとでは R_f 値が極めて接近しており，ペーパークロマトグラフィによる識別は不可能に近いが，今回われわれは硼砂水溶液中での沪紙電気泳動法によって両者の判別が可能なことを見出した．(東京教育大学理学部植物学教室)

Choline Sulfate Ester as an Intermediary Substance in Sulfur Metabolism of Fungi

by Michiko ITAHASHI*

Received December 14, 1959

Woolley and Peterson[1]) were the first to discover the presence of choline sulfate ester, $(CH_3)_3N^+CH_2CH_2OSO_3^-$, in the mycelium of *Aspergillus sydowi*. Recently, this finding has been followed by the detection of this substance in mycelial extracts of *Aspergillus oryzae*[2]) and *Penicillium chrysogenum*[3]).

In the previous paper[4]), the present author reported the effectiveness of choline sulfate ester as sulfur source in twenty-nine fungi, belonging to Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti. These findings indicate the possibility that this organic sulfate ester is a normal intermediate of sulfur metabolism in these fungi.

In the present study, it was found that choline sulfate ester is actually formed as an intermediate of assimilation of inorganic sulfate in various mould forms. The experimental results obtained will be briefly described in the following.

Experimental Methods and Results

The synthetic media used are as follows:

Stock solution: Basal Medium-1 (BM-1): One liter containing; 50 g. sucrose, 10 g. NH_4NO_3, 2g. $MgCl_2 \cdot 6H_2O$, 0.2 g. KH_2PO_4 and 0.01 g. $FeCl_3$. Basal Medium-2 (BM-2): One liter containing; 80 g. sucrose, 2 g. $(NH_4)_2HPO_4$, 2 g. KH_2PO_4, 1 g. $MgCl_2 \cdot 6H_2O$, 0.2 g. $CaCl_2$ and 0.01 g. $FeCl_3$.

Culture Medium: S^{35}-labeled inorganic sulfate (final concentration 0.005 M) was added to the basal media, BM-1 and BM-2, and the pH values were adjusted to 5.5 and 6.8, respectively. The total radioactivity (S^{35}) of these media, referred to in the following as Medium-1 (M-1) and Medium-2 (M-2), was about 30 μc per liter.

In a typical experiment, 50 m*l*. each of M-1 was accurately measured into six flasks (300 m*l*., conical) plugged with cotton, and sterilized in flowing steam for 30 min. on three successive days. The flasks were then inoculated with a pure culture of *Aspergillus niger*, and incubated at 28° for four days. The mycelium from each flask was separated by filtration on a Buchner funnel, and washed three times with 5 m*l*. of cold water. The mycelium was then dried to constant weight *in vacuo* and weighed. The dried mycelium thus obtained from 300 m*l*. of culture medium amounted to 2106 mg., which was homogenized in a mortar and 50 m*l*. of water was added. The mixture was transferred into a beaker and boiled for 20 min. The insoluble cell material was removed by filtration and the obtained filtrate was adjusted to pH 5. Inorganic sulfate ion was then removed as $BaSO_4$ by the addition of $BaCl_2$. To this solution, an equal volume of ethanol was added. After removal of the precipitate formed, the supernatant was concentrated to about 5 m*l*. under reduced pressure. In the one-dimensional paper chromatography, the sample to be tested was placed upon a filter paper strip (Toyo No. 50), together with S^{35}-labeled choline sulfate ester for the purpose of comparison, and allowed to run overnight in one of the following solvents: Solvent (1), an upper layer of *n*-butanol-acetic acid-water

* Aichi Gakugei University, Higashi-ku, Nagoya, Japan.

(5 : 1 : 4, v/v). Solvent (2), an upper layer of *n*-butanol–pyridine–water (4:1:2, v/v.) Solvent (3), *n*-propanol–water (7:3, v v). The obtained chromatogram was cut into segments. The radioactivity in each segment was measured with a Geiger-Müller counter. In each chromatogram there appeared a single peak of radioactivity, corresponding to R_f-values of 0.16 in Solvent (1), 0.10 in Solvent (2), 0.43 in Solvent (3), which was identified as choline sulfate ester (see Figs. I-III). The radioactivity values (in this paper) were corrected for the back ground counts.

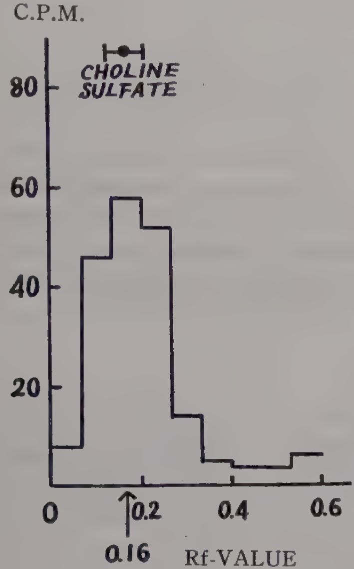


Fig. I

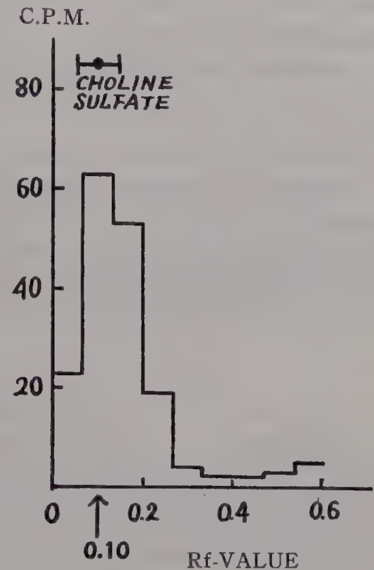


Fig. II

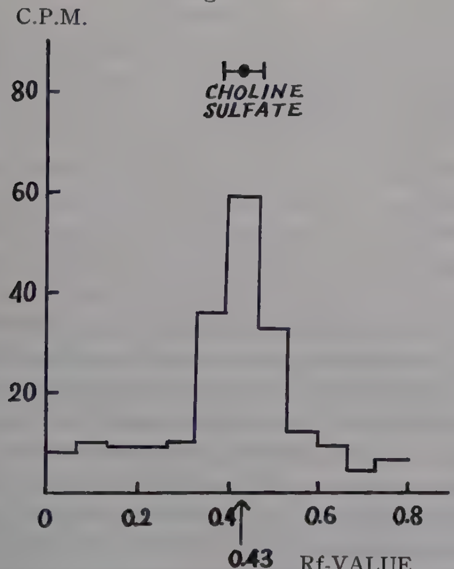


Fig. III

Fig. I-III. Chromatogram of hot-water extracts of *Aspergillus niger* grown in a synthetic medium containing S^{35}-labeled inorganic sulfate. The location of a spot for S^{35}-labeled choline sulfate ester is indicated at the top of the graph for the purpose of comparison. Solvents used: *n*-butanol: acetic acid: water (5 : 1 :4, v/v) for Fig. I, *n*-butanol: pyridine: water (4 : 1:2, v/v) for Fig. II and *n*-propanol: water (7:3, v/v) for Fig. III.

Table 1 shows the results obtained in the same way as above with five species of moulds belonging to Aspergillales as test organism. With each fungus examined, the formation of choline sulfate ester was confirmed in the mycelium.

Table 1

Organisms*	Weight of dry mycelium (mg.)	Incubation period (days)	Presence of choline sulfate ester formed
Aspergillus niger (Strain 4407)	2106	4	+
Asp. sydowi (〃 4402)	2570	4	+
Penicillium notatum (〃 4640)	1698	4	+
P. chrysogenum (〃 4626)	1892	4	+
Monascus purpureus (〃 4513)	1376	6	+

* Obtained from the Institute for Fermentation, Osaka (Table 1–2).

Table 2 shows the results obtained with *Neurospora sitophila*, two species of yeasts, six species of Fungi Imperfecti and two species of *Rhizopus*. Fifty m*l*. each of M-2 solution were poured into six flasks, sterilized, inoculated with fungal mycelium to be tested and incubated at 28°. The formation of choline sulfate ester during an incubation period was detected in all these fungi with the exception of *Saccharomyces* Strain XII and *Rhizopus nigricans*.

Table 2

Organisms*	Weight of dry mycelium (mg.)	Incubation period (days)	Presence of choline sulfate ester formed
Neurospora sitophila (Strain 6070)	1884	4	+
Saccharomyces Strain XII (〃 2113)	756	3	−
Hansenula anomala (〃 4540)	804	3	+
Alternaria tenuis (〃 4026)	2553	5	+
Botrytis cinerea (〃 5946)	3390	5	+
Fusarium solani (〃 5893)	1268	4	+
Oospora lactis (〃 4597)	1621	4	+
Pullularia pullulans (〃 4464)	1986	5	+
Trichothecium roseum (〃 6157)	1110	5	+
Rhizopus nigricans (〃 5411)	1293	4	−
Rhizopus oryzae (〃 4746)	1365	4	+

Cowie *et al.*[5]) has discovered, in the case of *Escherichia coli* (wild type), that the uptake of sulfate is suppressed by the intermediates supposed to be formed during the conversion of sulfate to organic sulfur compound. An experiment was therefore made to test if there is a competition between choline sulfate ester and inorganic sulfate with respect to their incorporation as sulfur sources; *Aspergillus sydowi* was used as test organism. A BM-1 solution containing S^{35}-labeled inorganic sulfate (0.005 M, final concentration; 0.3 μc per liter; pH 5.5) was prepared. 50 m*l*. of this medium and 50 m*l*. of another medium which contains, in addition, non-radioactive choline sulfate ester (0.005 M) as one more component, were transferred into 300 m*l*. conical flasks respectively, and each of them was inoculated with a culture of *Aspergillus sydowi*. The change in radioactivity was followed with 1 ml. aliquotes of the culture media removed at intervals. The results obtained are shown in Fig. IV. In the medium containing S^{35}-labeled inorganic sulfate as the sole sulfur source, a

remarkable decrease in radioactivity was observed. On the other hand, in the medium containing both S^{35}-labeled inorganic sulfate and non-radioactive choline sulfate ester, practically no decrease in radioactivity was detected, thus indicating that the consumption of inorganic sulfate was markedly suppressed in this medium.

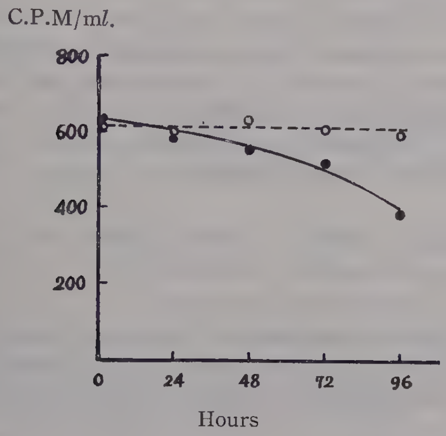


Fig. IV. Changes in radioactivity (S^{35}) of culture media (28°) with *Aspergillus sydowi*.

—●— radioactivity of medium containing S^{35}-labeled inorganic sulfate as the sole sulfur source.

- - -○- - - radioactivity of medium containing S^{35}-labeled inorganic sulfate and non-radioactive choline sulfate ester as sulfur sources.

Discussion

In the previous paper, the author has reported that, among the twenty-nine species of fungi studied, those belonging to Aspergillales, Pyrenomycetes and Fungi Imperfecti are capable of utilizing choline sulfate ester as sulfur source, while most of the fungi belonging to the yeast family, Phycomycetes, and Basidiomycetes do not utilize the organic sulfate ester. In some of the fungi which utilize choline sulfate ester, this substance has been identified as a normal constituent[1-3]).

In the present study it was discovered that this substance is produced from inorganic sulfate by the mycelia of two species of *Aspergillus*, two of *Penicillium* and one of *Monascus*, all belonging to Aspergillales, and capable of utilizing choline sulfate ester as sulfur source (see Table 1). The presence of choline sulfate ester in the mycelium has thus far been reported only in those belonging to Aspergillales, in which this organic sulfate ester is considered to be a normal intermediate of sulfate assimilation. In this connection, the above-described results (Table 2) indicating the presence of choline sulfate ester also in various fungi other than Aspergillales, will be of some interest. For two species of yeasts studied, this ester formation was recognized in *Hansenula anomala*, but not in *Saccharomyces* Strain XII. With two species of *Rhizopus* belonging to Phycomycetes, a positive result was observed in *Rhizopus oryzae*, but it was negative in *Rhizopus nigricans*. These results agree with the inference stated in the previous paper that choline sulfate ester is a normal intermediate in the sulfate assimilation in fungi which utilize this ester as sulfur source.

It has been shown in the foregoing paper, that *Alternaria tenuis* is the only organism in which, under certain experimental conditions, added choline sulfate ester is partially hydrolyzed to liberate inorganic sulfate in the course of incubation. Thus the isotopic confirmation of the production of choline sulfate ester from labeled inorganic sulfate in the mycelium of this mould seems to be of some interest.

When both inorganic sulfate and choline sulfate ester were simultaneously given to *Aspergillus sydowi* as sulfur sources, the preferential uptake of the organic sulfate

suppressed the incorporation of inorganic sulfate (Fig. IV), indicating that choline sulfate ester is an intermediary metabolite in the sulfate assimilation.

Summary

Using S^{35} as tracer, the formation of choline sulfate ester was detected in the mycelia of the following organisms: five species of fungus belonging to Aspergillales: *Aspergillus niger*, *Asp. sydowi*, *Penicillium notatum*, *P. chrysogenum*, and *Monascus purpureus*; *Neurospora sitophila* (Pyrenomycetes); *Hansenula anomala* (yeast); six species belonging to Fungi Imperfecti: *Alternaria tenuis*, *Botrytis cinerea*, *Fusarium solani*, *Oospora lactis*, *Pullularia pullulans*, and *Trichothecium roseum*; *Rhizopus oryzae* (Phycomycetes).

In *Aspergillus sydowi*, the preferential uptake of choline sulfate ester and the suppressing effect on the uptake of inorganic sulfate were observed.

In conclusion the author wishes to express her sincere thanks to Prof. F. Egami (University of Tokyo) and Prof. T. Mori (Nagoya University) for their kind guidance throughout the present study and for their kindness in reading the manuscript.

References

1) Woolley, D. W., and Peterson, W. H., J. Biol. Chem. **122**: 213 (1937). 2) Itahashi, M., Bull. Aichi Gakugei Univ. **4**: 37 (1954). 3) de Flines, J., J. Amer. Chem. Soc. **77**: 1676 (1955). 4) Itahashi, M., Bot. Mag. Tokyo **72**: 275 (1959). 5) Cowie, D. B., Bolton, E. T., and Sands, M. K., J. Bact. **62**: 63 (1951).

Addendum

In course of printing, the author found out that A. Ballio *et al.* had carried out the similar investigations. Ballio, A., Chain, E. B., Dentice di Accadia, F., Navazio, F., Rossi, C., and Ventura, M. T., Selected Scientific Papers from the Istituto Superiore di Sanità **2**: 343 (1959).

摘　　要

菌類における硫黄代謝の中間生成物としてのコリン硫酸エステル

板　橋　美　智　子

Aspergillales に属する *Aspergillus niger*, *Asp. sydowi*, *Penicillium notatum*, *P. chrysogenum*, *Monascus purpureus;* Pyrenomycetes に属する *Neurospora sitophila;* 酵母の一種である *Hansenula anomala;* 不完全菌類に属する *Alternaria tenuis*, *Botrytis cinerea*, *Fusarium solani*, *Oospora lactis*, *Pullularia pullulans*, *Trichothecium roseum;* 藻菌類に属する *Rhizopus oryzae* を S^{35} で標識された無機硫酸を含む合成培地で培養し，いずれの場合にも菌体内にコリン硫酸エステルの生成を認めた.

Asp. sydowi に硫黄源として無機硫酸とコリン硫酸エステルとを同時に与えた場合，後者によって前者の吸収が抑制された.（愛知学芸大学名古屋分校）

アメリカアリタソウの春化処理

(1) ことなった処理温度が発育ならびに精油含量におよぼす影響について*

大橋　裕**・市川郁雄***

Hiromu OHASHI** and Ikuo ICHIKAWA***: A Study of Vernalization on American Wormseed (1) The Influence of Various Temperatures on its Development and Yield

1959年9月25日受付

アメリカアリタソウ *Chenopodium ambrosioides* L. var. *anthelminticum* (L.) A. GRAY は熱帯アメリカ原産の多年生植物である．この植物の果穂をつけた地上部を水蒸溜してえた精油はヘノポジ油 chenopodium oil とよばれ，駆虫薬としてもちいられる．精油は果実，葉，茎にふくまれているが，とくに果実に多い[1−4]．

この植物の春化処理† は 1955†† ～57 年の3年間におこなったが，ここでは 57 年度の実験結果を中心として報告する．

I. 材料と方法

種子は武田薬品工業株式会社研究所京都試験農園より分与をうけたものをもちい，栽培は長崎大学薬学部附属薬草園（長崎市）でおこなった．

春化にさいしては，処理中の種子の発芽をできるかぎりさけることがこのましい．そのため，春化にさきだち，下記の2つの実験をおこなった．

（1） 種子の発芽と温度の関係．

リーベンベルヒ発芽試験器をもちい，15°，20°，25° および 30° で発芽試験をおこなった（表 1）．

表1 発芽試験

温度 °C	発芽開始までの日数	発芽継続日数	発芽勢 %	発芽率 %
15	—	—	0	0
20	5	1	1	1
25	4	14	55	75
30	—	—	0	0

種子消毒，0.1％ウスプルン 20 分間；置床粒数，各 200 粒；発芽勢，13 日間の発芽率；発芽率，18 日間の発芽率．

発芽しうる温度の範囲はせまく 15° 以下又は 30° 以上で春化するときは，処理中の種子の発芽を心配する必要はないが，十分に吸水せしめた種子を発芽の適温の 25° 前後で春化するときは，4 日後には発芽を始めると推定せられる．

（2） 胞果および種子吸水量の浸水時間にともなう変化ならびに最大吸水量

一般にアメリカアリタソウにおいて，種子とよばれ播種にもちいられているものは，実際は「萼につつまれた胞果 utricle」（以下たんに胞果とよぶ）で

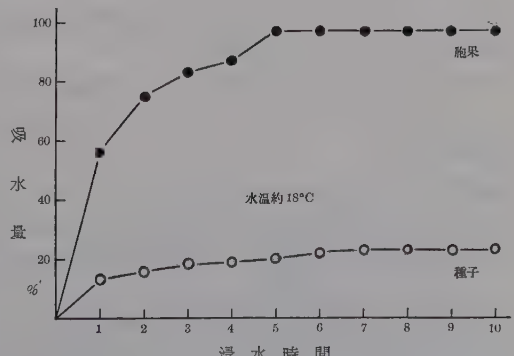


図1　胞果・および種子吸水量の浸水時間にともなう変化

* 薬用および油料植物の春化処理　第6報

** 長崎大学薬学部生薬学教室 Institute of Phamacognosy, Faculty of Pharmacy, Nagasaki University, Nagasaki, Japan.

*** 長崎市中央保健所試験室 Service Section, Nagasaki Municipal Health Center, Nagasaki, Japan.

† Khlebnikova, N.A., Moskovets, K.C.[5] により 1941 年におこなわれているが原報にせっすることができなかったので，詳細は不明．

†† 55 年度の実験結果は日本薬学会九州支部第3回例会講演発表 (1956).

表 2　発　　芽

処　　理	35°	25°	15°	5°	対　　照
発芽始月・日	Ⅳ, 9	Ⅳ, 6	Ⅳ, 6	Ⅳ, 7	Ⅳ, 8
平均発芽日月・日	Ⅳ, 9	Ⅳ, 9.1±0.27	Ⅳ, 8.2±0.20	Ⅳ, 9.0±0.27	Ⅳ, 9.2±0.29
同促進日数	0.2	0.1	1.0**	0.0	
発芽揃月・日	Ⅳ, 9	Ⅳ, 15	Ⅳ, 16	Ⅳ, 19	Ⅳ, 19
発芽期間日	0	9	10	12	11
発芽率　%	1.0	63.0	81.5	77.5	66.0

置床種子数，各 200 粒;発芽始，始めて発芽をみた日，25° 処理および 15° 処理は置床時(4 月 6 日) すでに発芽していたので発芽始を 4 月 6 日とした；平均発芽日，各種子が発芽した日の平均値；発芽揃，発芽率が最高にたっした日；発芽期間，発芽始から発芽揃にいたる日数；発芽率，置床種子にたいする発芽粒数%；**，対照にたいし危険率 1 %で有意差あり.

ある．胞果の重量は 1000 粒重 0.44～0.49 g (平均 0.46 g) で，おなじく種子は 0.32～0.35 g (平均 0.33 g) である．両者の重量の差は，主として萼の重量と考えられる.

春化にはふつう播種にもちいられる胞果をもちいることにした．春化処理時の胞果吸水量を決定するために，胞果および種子を浸水し，吸水量の時間的変化ならびに，その最高量を測定した (図 1).

測定方法は胞果ならびに種子各 1 g をとり，小ビーカー (容量 20 cc) に入れ，水約 5 cc をくわえて水浸し，1 時間おきにとりだして，表面に附着している水を沪紙で吸いとり，重量をはかり，風乾重にたいする吸水率を算出した.

(3)　春化のやり方

胞果 5.0 g をとり，ウスプルン 0.1%液に 60 分間ひたして消毒後，2 回水洗し，径約 8 cm のシャーレに入れ，吸水量が風乾重の 100%になるように水をくわえて，約 25° の恒温槽中にて 24 時間催芽した．催芽後，35° (34.9±1.56°)，25° (25.7±0.58°)，15° (14.8±0.25°)，5° (4.9±0.47°) の恒温槽中にて，それぞれ 5 日間春化した.

対照としては吸水催芽のみをおこなった胞果をもちい，春化を終了した胞果と同時に，4 月 6 日に苗床に播種した*.

播種時の胞果の状態は 25° 処理および 15° 処理において，若干発芽しているものがみとめられたのみで，他の処理，対照においては発芽はみとめられなかった.

(4)　栽培管理

苗は 6 月 24 日，本圃に条間 60 cm，株間 30 cm に移植した．植付けは 1 区 14 本 3 回反覆の任意配列法によった．肥料は基配として 1 a あたり堆肥 50 kg，硫安 2 kg，過燐酸石灰 2 kg，塩化カリ 1 kg をあたえた.

II. 実験結果および考察

(1)　発　　芽

春化終了後，胞果をリーベンベルヒ発芽試験器に置床し，発芽最適温の約 25° の恒温槽に入れ，春化が発芽におよぼす影響をしらべた (表 2).

発芽の促進は，15° 処理において約 1 日はやめられたのみで，他の処理，対照間にはあきらかな差はみとめられなかった．これにひして，発芽率は春化により大きな影響をうけ，とくに 35° の高温で処理した種子はほとんど発芽しなかった．25° 処理の発芽率は対照とほとんどかわらないが，15° 処理および 5° 処理は対照より 10% 以上もよくなった.

苗床における発芽は，25° 処理および 15° 処理は 4 月 10 日，5° 処理は 12 日，35° 処理および対照は 13 日にはじまり，35° 処理をのぞき，春化処理した種子の発芽はいくぶんはやめられた．35° 処理の発芽率は非常にわるかったが，多量の種子をまきつけていたので，かろうじて移植し栽培するだけの苗がえられた.

(2)　草丈の伸長

草丈の伸長にかんしては，とくに伸長の初期にあるていどの差がみとめられた．35°，25° および 15° 処理の草丈はいずれも移植時より 7 月中下旬までは

* 参考のために播種後 5 日間の平均気温 (午前 10 時測定) をあげると次の通り.

月・日	Ⅳ, 7	8	9	10	11	平均
気温 °C	15.8	16.9	19.0	19.0	20.2	18.2

表 3 草丈の伸長 (cm)

月日＼処理	35°	25°	15°	5°	対照
VII, 9	33.6 (112.0)	38.3 (127.7)	34.1 (113.7)	26.5 (88.3)	30.0 (100.0)
VII, 15	43.8 (109.5)	49.4 (123.5)	43.4 (108.5)	36.0 (90.0)	40.0 (100.0)
VII, 30	82.3 (104.6)	86.8 (110.3)	85.1 (108.1)	75.5 (96.1)	78.7 (100.0)
VIII, 6	89.3 (97.4)	92.6 (101.0)	96.2 (104.9)	88.0 (96.0)	91.7 (100.0)
VIII, 16	96.1 (97.2)	100.3 (101.4)	103.6 (104.8)	98.8 (99.9)	98.9 (100.0)
VIII, 27	100.4 (97.6)	103.9 (101.0)	104.8 (104.8)	100.1 (88.8)	102.9 (100.0)
IX, 5	100.8 (97.6)	104.1 (100.9)	107.0 (103.7)	103.9 (100.7)	103.2 (100.0)

( ) は対照にたいする草丈の相対値.

対照よりたかかったが，この3者のうちで25°処理がもっともすぐれていた．これにたいして，5°処理は生長があまりよくなく，7月下旬ないし8月中旬までは対照より低い価をしめした.

(3) 開　花

春化が発育におよぼす影響をあきらかにするために，開花状態をしらべ，図2ならびに表4にしめした.

35°および25°処理は平均して約4日の開花の促進があきらかにみとめられた．15°処理と対照の間には有意な差はみとめられず，5°処理はぎやくに開花がおくれた.

以上の結果から，アメリカアリタソウは温度発育段階の通過にさいして，すくなくとも35°ないし25°の高温を要求すると推察される.

川谷，大野[6,7]は，アメリカアリタソウの栽培，とくに冷涼地である長野県高冷地，東北および北海道における栽培実験の結果，冷涼地におけるこの植物の発育は，暖地の埼玉県春日部における栽培にひして，遅延することをあきらかにした．この原因は暖地に栽培された植物は冷涼地の植物にひして，播種後，温度発育段階の通過にてきした高温に遭遇する機会がおおく，この段階をよりすみやかに経過しうることに一因があるのではないかとおもわれる.

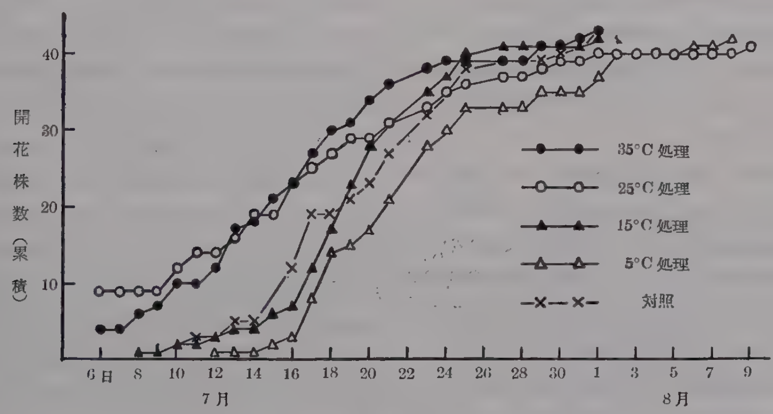


図 2 開　花

表 4 開　花

処　理	35°	25°	15°	5°	対　照
開花始月・日	VII, 6	VII, 6	VII, 8	VII, 12	VII, 9
平均開花日月・日	VII, 15.9±2.14	VII, 16.2±2.63	VII, 18.6±1.39	VII, 22.9±1.96	VII, 20.1±1.72
同促進日数	4.2**	3.9*	1.5	−2.8*	
開花揃月・日	VIII, 1	VIII, 9	VIII, 1	VIII, 8	VIII, 1

開花始，始めて開花をみた日；平均開花日，各株が開花した日の平均値；開花揃，全株開花した日；**，対照にたいし危険率1%で有意差あり；*，おなじく危険率5%で有意差あり.

表5 精油含量その他

処理	35°	25°	15°	5°	対照
草丈 cm	109.4±5.39	112.3±5.21	114.5±5.27	111.0±3.90	109.5±4.79
地上部風乾重 g	85.8±12.34	85.2±9.95	79.2±9.07	79.9±14.82	72.9±7.41
胞果風乾重 g	43.7±6.69	42.2±5.99	39.0±5.05	40.0±7.45	38.0±4.42
胞果中精油含量%	0.87±0.018**	0.84±0.062*	0.90±0.077**	0.78±0.064	0.79±0.018
精油中アスカリドール含量%	71.23	71.41	71.27	71.41	71.60

**, 対照にたいし危険率1%で有意差あり; *, おなじく危険率5%で有意差あり.

(4) 胞果中の精油含量, その他 2, 3 の形質について

9月 27 日, 各処理, 対照同時に収穫し, 若干の形態測定をおこなうとともに, 胞果中の精油の含量, ならびに精油中のアスカリドール ascaridole の含量を定量した.

表5にみられるように, 草丈, 地上部風乾重, 胞果風乾重は各処理, 対照内の個体間の変動が大きく, 各処理, 対照間には有意な差はみとめられない.

胞果中の精油含量は, 1回に 50 g の試料をとり, それぞれ3回, 局方精油定量器 (比重1以下)[8] をもちいて定量した. 15° 処理は含量もっとも多く, 対照にたいして 10% 以上も増加し, さらに 35° 処理, 25° 処理においても, それぞれ数%の含量増加がとめられた. これにたいして 5° 処理の含量は対照とかわらなかった.

15° 処理において精油含量が増加することは, 55 年度の実験においても, あきらかにみとめられ, とくに 10 日間処理したものは非常に含量がたかまり対照にたいし約 26%, おなじく 20 日処理は約 11 %ふえた. (20 日処理の胞果中精油含量 1.93%, 10 日処理 2.20%, 対照 1.74%).

さらに, 上記の精油の定量とは別個に, できるかぎり多量の精油を抽出して, 精油中のアスカリドールの含量をイギリス薬局方記載の方法[9]により定量したが, 差はほとんどみられなかった. 春化により胞果中の精油含量は変化するが, 精油の質には影響をおよぼさないようである.

以上の結果より, アメリカアリタソウを約 15° の温度で, とくに 10 日間前後春化することは, 植物体中精油の大部分を含有する胞果の量に影響したり, また精油の質を変化せしめることなく, 胞果中の精油含量を増加せしめ, ひいては1株または単位栽培面積あたりの精油収量の増加をもたらし, 生産上有利であると考えられる.

(5) 考察 とくに春化による成分含量変化の過程について

アメリカアリタソウの胞果中の精油含量は, 植物の発育がすすむにつれて, すなわち果実が成熟するにともなって, 増加することがしられている[4,10].

しかして, 本研究においては, 春化により開花がはやめられた植物は, 当然収穫時の果実の成熟も対照植物よりすすんでいたものと考えられる. ところが, それらを材料として成分の含量を定量し相互に比較したので, 発育状態のことなった植物からえられた成分含量を比較したことになる.

よって, 対照にたいして発育の促進がみとめられた 35° 処理や 25° 処理における胞果中の精油含量の増加は, 発育の促進にともなった現象ではないかと考えられる. この過程は次のようになる.

(1) 春化⟶発育促進⟶成分含量変化

ところが, 15° 処理においては, 対照にたいする発育の促進はみとめられないにもかかわらず, 最大の精油含量の増加がみとめられた. このことは非常に興味があり, 春化による成分含量の増加は, (1) の過程とは無関係に, 処理温度の直接的な効果として生じ, 次のような過程があきらかに存在しうることを示唆している.

(2) 春化⟶成分含量増加

さらに, (1) のようなばあいにも, 実際には (2) の変化もくわわったのではないかと推測せられ, 次のような成分変化過程の存在の可能性も十分に考えられる.

(3) 春化⟶成分含量増加; 春化↘発育促進↗成分含量増加

以上のことより, 植物の発育初期の環境温度, いいかえれば, 植物が温度発育段階の経過にさいして

経験する温度は，植物の発育にたいして重要な関係があるばかりでなく，その中にふくまれている特定の成分含量にたいして，あるていどの直接的な影響力，支配力を有しているものとおもわれる.

さらにこの現象は，たんに春化にさいしてのみみとめられる現象ではなく，自然に生育している植物においても，おこっている現象ではないかと推測せられる.

III 要　約

アメリカアリタソウの萼につつまれた胞果（一般に種子とよばれ播種にもちいられる）に，その風乾重の 100% に相当する水をふくませて，約 25° の温度で 24 時間催芽後，約 35°，25°，15° および 5° の温度でそれぞれ 5 日間春化し，吸水催芽のみをおこなった胞果を対照として，1957 年 4 月 6 日，同時に播種した.

1) 35° 処理はほとんど発芽しなくなり，25° 処理の発芽率は対照とほぼひとしく，15° 処理および 5° 処理の発芽率は対照より 10%以上も増加した．また 15° 処理の発芽は他の処理，対照より約 1 日はやまった.

2) 35° 処理および 25° 処理は約 4 日，対照より早く花が咲いたが，15° 処理の開花は対照とかわらず，5° 処理の開花は対照より約 3 日おくれた．よって，アメリカアリタソウは，すくなくとも 35°〜25° の比較的高温で，その温度発育段階を通過しうるとかんがえられる.

3) 1 株あたりの収果量はかわらないが，果実中にふくまれている精油含量は春化により大きな影響をうけた．35° 処理および 25° 処理は対照にたいし数%，15° 処理は対照にたいし 10% 以上増加したが，5° 処理と対照間には差はみとめられなかった．精油中のアスカリドール含量においては，各処理，対照間には差はみとめられなかった.

なお，15° 前後の温度で 10 日間および 20 日間春化した 1955 年度の実験によると，精油含量は 10 日処理は対照にたいし 26%，20 日処理は 11% 増加した.

以上の結果より，アメリカアリタソウを 15° 前後の温度で，とくに10日間春化することは，精油収量の増加をもたらし，栽培上有利であると考えられる.

また，植物の温度発育段階における温度は発育に関係するのみでなく，その成分含量にたいしても直接的な大きな影響力を有するらしい.

謝辞　本文を擱筆するにあたり御校閲をいただいた北海道大学理学部松浦一，宇佐美正一郎両教授にたいして深く感謝します．さらに栽培管理を熱心におこなって下さった長崎大学薬学部南里卯吉氏，ならびに成分の定量に御協力をいただいた佐々田昭七氏にお礼を申上げます.

文　献

1) 刈米達夫・渥美峻次郎・照井留吉，薬誌，**40**：736 (1920). 2) 刈米達夫・木村雄四郎，薬誌，**41**：921 (1921). 3) 若林栄四郎・木村雄四郎，薬誌，**44**：722 (1924). 4) 川谷豊彦，(佐々木喬監修) 綜合作物学（全六巻）工芸作物篇，嗜好料の部薬用の部 369 (1953). 5) Khlebnidova, N. A., and Moskovets, K. G., Compt. Rend. Acad. Sci. U.S.S.S. **32**：161 (1941); C.A. **37**：1012 (1943). 6) 川谷豊彦・大野忠郎，薬誌，**72**：1478 (1952). 7) 川谷豊彦・大野忠郎，薬誌，**73**：1044 (1953). 8) 縮刷第 6 改正日本薬局方註解，1173 (1959). 9) British Pharmacopeia, 134 (1953). 10) 岩佐準二・安田美也子，生薬学雑誌，**78**：55 (1953).

Summary

American wormseed (*Chenopodium ambrosioides* L. var. *anthelminticum* (L.) A. GRAY) has medicinal use and is also a source of essential oil (chenopodium oil), 45–70 percent of which is ascaridole.

American wormseed seeds (in fact, they are utricle), vernalized at four temperatures, 35°, 25°, 15° and 5° for 5 days respectively, were sowed on April 6, 1957, using as the control non-treated seeds.

1) The germination, especially the germination rate, was greatly influenced by the vernalization: the 35°-treated group hardly germinated, the germination rate of the 25°-treated group was generally equal to the control. But those of the 15°-treated and of the 5°-treated

group increased over 10 percent more than the germination rate of the control.

2) To clarify the effect of the vernalization on the plant development we examined the flowering season. The 35°-treated group and the 25°-treated one flowered about 4 days earlier than the control, but the flowering of the 15°-treated one was not different from that of the control, and that of the 5°-treated one was a little later than that of the control. Therefore American wormseed seems to pass through its vernalization phase at comparatively high temperatures, from 25° to 35°.

3) As to the yield of air dried seeds, there was no difference between the treated groups and the control, but the content of essential oil in seeds was greatly influenced by the vernalization: that of the 35°-treated group and the 25°-treated one increased several percent and the 15°-treated one more than 10 percent, but that of the 5°-treated one less than the control. As for the content of ascaridole in essential oil, there is no difference between the treated ones and the control.

According to the experiment in 1955 on seeds that were vernalized at 15° for 10 days and for 20 days, the content of essential oil in seeds increased 26 percent in the 10-day-treated group and 11 percent in the 20-day-treated groups, as compared with the control.

From these effects, American wormseed vernalized at 15°, especially for about 10 days results in an increase in the yield of essential oil and is useful in cultivation. And the temperature in the vernalization phase of plants is not only related to development, but also seems to have great influence upon the content of the active constituent.

蘚類および羊歯類数種の細胞における硝酸銀還元反応の検討*

吉　田　吉　男**

Yoshio YOSHIDA**: Some Critical Information on the Silver-nitrate-reduction in the Cells of Several Musci and Ferns.*

1959 年 10 月 22 日受付

種々の生体組織にはいちじるしい硝酸銀還元能がみられることはかなり古くから注目され，今日ではその還元要因はアスコルビン酸 (Vitamin C) の局在のせいであるとされ，さらにこの反応は逆にアスコルビン酸の組織化学的検出法[1,2]として広く応用されている．植物細胞の場合には特に生きている葉緑体にこの反応の強いこと (いわゆる Molisch 反応[3])) が注目され，多くの研究がなされている．しかしこの反応にもなおより慎重な検討を要する点も少なからず，すでに前報[4])において *Elodea* その他の数種の沈水植物細胞について検討した結果，硝酸銀還元反応がすべての場合に絶対的にアスコルビン酸の存在や局在を示すものといえるかどうかその結果の組織化学としての判定には慎重な考慮を必要とする場合もあるのではないかということを主張したが，引続いて今回は従来取上げられることの少なかった蘚および羊歯類の数例について同様の関係を検討した結果について報告する．

材料と方法

材料： *Catharinaea undulata* Web. et Mohr. [ナミガタタチゴケ], *Funaria hygrometrica* Sibth. [ジメリヒョウタンゴケ], *Mnium vesicatum* Besch. [オオバチョウチンゴケ], *Plagiothecium nemorale* (Mitt.) Jacq. [ミヤマサナダゴケ], *Selaginella japonica* Miq. [クラマゴケ], *S. uncinata* Spring. [コンテリクラマゴケ] の 4 種の蘚類と 2 種の羊歯類の葉を被験材料とした．

なおクラマゴケ類は細胞間隙内の空気の存在によりいちじるしく鏡査が妨げられるからこれらはあらかじめ水流ポンプによる真空浸潤法を行なって蒸溜水と置換したものを検査に供した．

試薬： 種々の pH に調整した 10 %硝酸銀液をスライド上の葉の上に十分に滴下し細胞内の反応の様相を鏡査追求した．試薬の pH は酢酸および 5N アンモニア水を適量加えて pH≒3〜9 の種々の段階に調整した．この際沈澱を生ずることもあるがそれは沪過によって除去した．これらにより普通室内散光中での単純浸漬処理，暗黒中での処理，および火焔を通しての若干の加熱処理などの操作を各材料について実施した．

ペーパークロマトグラフィー： 前報[4])に記載したと全く同じ操作によって各植物中の還元性物質を検索した．

実験結果

一般的な反応の傾向としては高等植物における場合よりは反応の度合は弱いことが多いが，これらの 6 種の細胞においても前報[4])の結果とほぼ類似の関係が示される (Fig. 1)．すなわち一般にアンモニア性試薬では細胞全体にわたっての反応の一様分散および沈澱の生起がいちじるしい．ナミガタタチゴケはほとんど細胞内全体が全く暗黒化することも多い．しかし pH 値が低下するにつれて葉緑体に特異的に黒化反応が現われ大凡 pH≒5〜7 の範囲において一般に特異的な葉緑体の黒化反応が示され，pH≒5.6 附近に極大のみられることが最も多い．ただしナミガタタチゴケだけはさらにこれが酸性に傾いていて pH≒3.2 位の酢酸酸性において最も顕著であり，黒化の程度も他種にくらべて特に強い (Fig. 2. A. C)．他の 5 種では試薬の pH が 4〜3 となると葉緑体の特異的反応性はかえって低下す

---

* 要旨は第 24 回大会 (仙台) に報告．

** Department of Biology, Faculty of Science, Niigata University, Niigata, Japan. 新潟大学理学部生物学教室．

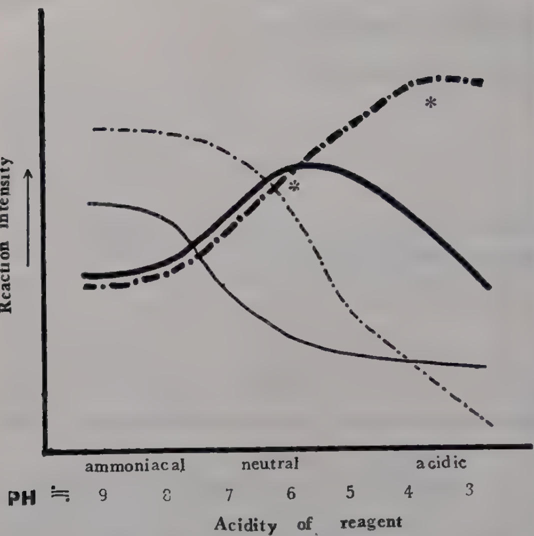


Fig. 1. Experiential diagram showing the relation between the reaction-intensity and acidity of silver reagent.

} Thick line: in chloroplasts of musci and *Selaginella*.

} Thin line: in cytoplasm and vacuoles of musci and *Selaginella*.

} Broken line: in *Catharinaea*.

* : So-called "Molisch's reaction" is typically shown.

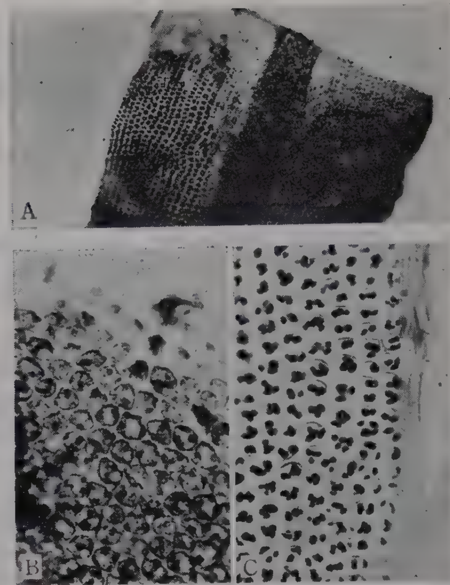


Fig. 2. Photographs showing the appearance of silver-nitrate-reduction in the leaf cells of *Catharinaea*.

A: Treated with acidic reagent (pH≒3.2). Reaction is faint or negative in necrotic zone, and typically strong in intact portion.

B: Treated with ammoniacal reagent. Darkening and precipitation are remarkable in a whole cell, but chloroplasts show negative reaction.

C: So-called "Molisch's reaction" occurred by acidic reagent. Note the localized blackening of the chloroplasts.

る．反応は早ければ処理後5分位から遅くも20〜30分以内には肉眼的にも識別可能となってくる．いずれの場合にも若干加熱することによって反応の速度および度合はいちじるしく促進される．また明処理と暗黒処理とでほとんど大きな差を認めることはなかった．

また，これらの材料では硝酸銀処理に際し細胞膜に顕著な褐変反応が現われることがあり，とくにミヤマサナダゴケ，オオバチョウチンゴケ，ナミガタタチゴケではそれが目立ち，葉緑体の特異的黒化反応の強くない時，この細胞膜褐変のきわ立つことが多かった．

葉緑体に特異的黒化を生ずる典型的な場合には，そのいわゆる Molisch 反応は健全細胞にのみ生じ，死滅細胞では全く反応は負であって生死両細胞のコントラストはきわめて顕著で，高等植物についての従来の知見と大凡一致している．しかし Molisch 反応が典型的でない条件の時には，この関係が必ずしもそう明瞭でない場合もあり，ことにミヤマサナダゴケでは死細胞でも完全に負であるとはいいきれなかった．ナミガタタチゴケでは pH≒3.2の酸性試薬できわめて典型的な Molisch 反応が生じその時には生死のコントラストはきわめて明瞭であって，直接に切断などによって死滅した細胞ではもちろん，その傷害による何らかの影響がおよんだと思われる数層の周辺の細胞でも，反応が弱まるか，または負であって，反応の度合が細胞の健全度に比例するという従来の知見と全く一致している (Fig. 2. A)．しかしながら試薬の pH が中性からアンモニア性へと傾くにつれて葉緑体特異反応は弱まり逆に細胞質全般に沈澱や一様暗黒化が強まってくるが (Fig. 2. B)，それにつれてこのような健全，傷害，死滅の関係もその差が次第に不鮮明となってきて，さらには前記の関係が逆転し，傷害部周辺のその影

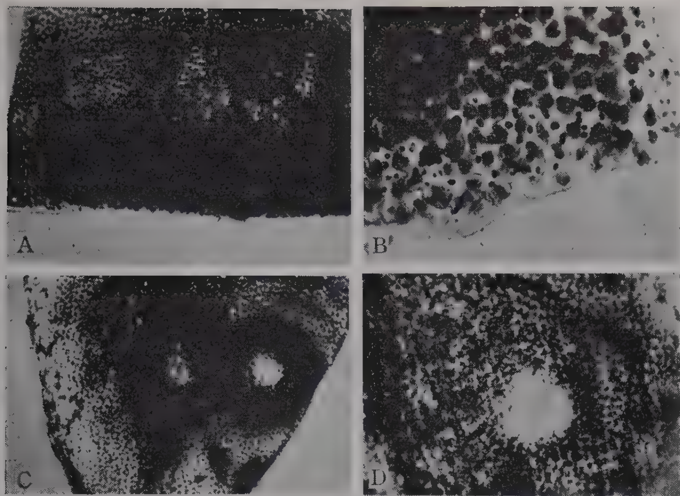


Fig. 3. Photographs showing the acceleration of silver-nitrate-reaction in necrobiotic zone of the leaf of *Selaginella* (pH of reagent is about 5.6).
A: A cutt-off-margin. C: Stabbed points with a needle. B and D: Magnified photographs of A and C, respectively. Note the remarkable blackening of chloroplasts.

響を直接に蒙っていると思われるところの，さきには全く反応の負であった部域が，かえって，むしろ，より敏感に細胞内全体の暗黒化を顕著に現わしていることすら注意される．このような傾向はシメリヒョウタンゴケ，およびコンテリクラマゴケでも認められ，これらでは葉緑体特異反応をもたらす条件下でも傷害部周辺における反応の促進が僅かに認められ，さらにクラマゴケにおいては，この現象がきわめて典型的にかつ顕著にみられる．すなわちクラマゴケが葉緑体特異反応を生ずるに最適の pH≒5.6 の試薬に浸漬しただけでは反応は普通やゝ緩漫であるが，葉の切断面，針刺点，さらにはピンセットではさんだだけでも，それら傷害部近辺の一定範囲内の細胞には，葉緑体の特異的黒化反応がきわめて速かにかつ強く起ってくる (Fig. 3)．その様相は丁度 dead ring (死環) と呼ばれる現象によく似ている．これはガラスナイフでの切断，白金針での刺殺でも全く同様であるから決して単に鉄器など金属の使用による攪乱でないことは明らかであって，この場合傷害の影響によって反応を促進すべき何らかの内的要因が生じているものと考えられる．直接に死滅した細胞自体では，やはり反応は負であってあらかじめ種々の手段で殺された葉では，硝酸銀還元は全く起らない．

オーバチョーチンゴケでは同一の葉でも部分によってかなり反応様相が違っていて，pH≒5.6 の試薬でも中肋および葉縁の細胞は全体の一様暗化が目立ち，中肋に近い葉身および葉尖部近辺では部分的に葉緑体の特異的黒化がひきたっていることがしばしば注意された．これは同一材料，同一処理でも部分によって反応生起のための内的要因が異なっていることを示している．

クロマトグラフィーによる還元性物質の追求の結果は大凡前報[4]の沈水植物細胞の場合と近似のスポットが示され，やはり，従来硝酸銀還元の主体であるとほぼ定説とされてきたアスコルビン酸の位置にではなく，同様にほぼ Rf 0.08 の近辺にやや弱いけれども明かな暗灰色のスポットが検出された (Fig. 4)．Rf 0.4 附近に出現すべきアスコルビン酸もほとんど判定に苦しむ程の微弱な痕跡的にその存在が認められるが，これらの場合，それが反応の主役を果しているとはとても思えず，この Rf 0.08 附近のスポットがこれらの蘚や羊歯等数例の細胞における硝酸銀還元反応の主体であることは明らかである．オオバチョウチンゴケ，ナミガタタチゴケ，ミヤマサナダゴケでは Rf 0.08 の主スポットに連接したスポットがあるが，これは前報の沈水植物細胞の場合に副次的とみられたスポットと対応するもの

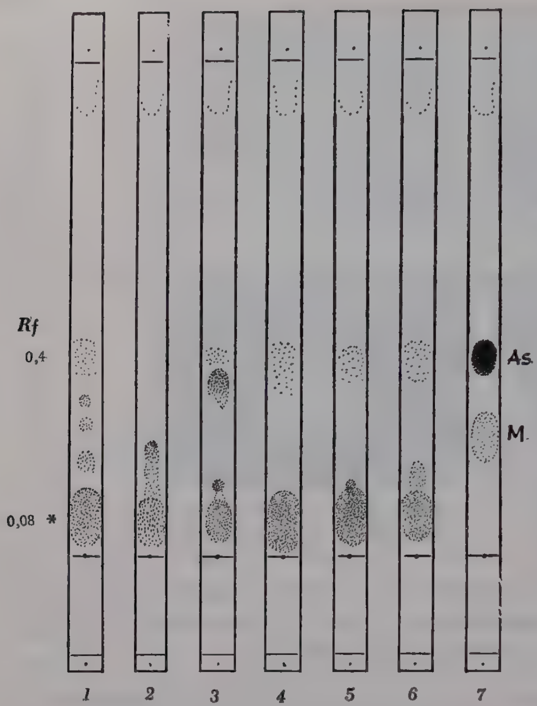


Fig. 4. Schematized paper chromatograms. (1), *Selaginella japonica*, (2), *S. uncinata*, (3), *Catharinea undulata*, (4), *Funaria hygrometrica*, (5), *Mnium vesicatum*, (6), *Plagiothecium nemorale*, (7), Pure ascorbic acid-solution with metaphosphoric acid. As: Ascorbic acid. M: Metaphosphoric acid. *: Unknown reducing agent.

かもしれない．これら以外にもクラマゴケ，コンテリクラマゴケ，およびナミガタタチゴケでは暗褐色のスポットが現われるが，共通のものはなく，また細胞膜褐変のいちじるしかった種とは必ずしも一致せず，その意義は不明である．

Rf 0.08 のこの主スポットが物質的に何であるかは今なお手がかりをつかめないでいるが，従来，定説とされたアスコルビン酸以外に，場合によっては，このように硝酸銀還元反応に主体をなす他の物質があるということは注目に値いしよう．

考　　察

アスコルビン酸（Vitamin C）は細胞内において水素伝達体その他として代謝過程に重要な機能を果し[5]，また光合成にも密接な関連をもつ[6,7]といわれ，その存在の検知は細胞生理学上，きわめて重要な意味をもつものである．これの細胞化学的検出法としては，従来 Giroud-Leblond 法[1]はじめ硝酸銀還元反応が広く応用されている[2]．永井 (1950—1954)[8] の広範な研究および優れた綜説に概説されているように生体細胞に顕著にみられる硝酸銀還元の能力が細胞におけるアスコルビン酸の存在に基ずくものであることは，今日ほぼ定説とされている．しかしながら，これらの反応をアスコルビン酸の細胞化学的検出法として適用するには，必ずしもすべての場合に，その特異性および敏感度が十分であるとはいいきれないことは，すでに Mirimanoff (1938)[9]，新家・重永 (1947)[10]，飯島・平岡 (1950)[11] および Danielli (1953)[12] らによって指摘されており，永井(1951)[8] もアスコルビン酸以外にもタンニン様物質，DOPA，フラボノイドなどが硝酸銀還元に関与している場合もあることを明らかにしている．著者もすでに前報[4]において数種の沈水植物細胞の場合には予想に反してアスコルビン酸とは全く違った別の物質によって還元が起されていることを報告した．蘚および羊歯類数種について検討した本報の結果もほぼ近似であって，硝酸銀還元がアスコルビン酸によってではなく前報[4]における と同様の未確認物質が主体となって起されていることが明らかとなった． 前報[4]に詳記したように，この場合安定剤として用いられるメタ燐酸自体も硝酸銀試薬に反応するが，これは比較試験では Rf 値は全くかけ離れている．永井 (1951)[8] の報告にも種々の物質が反応にあずかる場合もあるが，そのような場合も還元の主体はやはりアスコルビン酸が果しており，それらは副次的なものであるにすぎない．ただ，アオミドロの場合にタンニン様のスポットが現われた以外，アスコルビン酸が全く検出されなかったのが唯一の例外である．このスポットが物質的に何であるかは前報に報じた以外に手がかりはまだ得られていないが，いずれにせよ，このことは硝酸銀還元反応を直ちにアスコルビン酸の検出に適用するには，このような顕著な例外がある以上必ずしもその信頼性が確実ではないことを明示している．

従来，硝酸銀還元反応はアスコルビン酸の細胞内における局在性をも示すものであるといわれてきた

が，永井 (1950)[8]は反応生起の機構について見事なモデル実験その他から，それはむしろ液胞および細胞質中に一様に分散しているものであって，葉緑体の特異的黒化すなわちいわゆる Molisch 反応は葉緑体における一次的局在性を示すものではなく，還元銀膠質粒子が二次的条件によって葉緑体表面に吸着されて起るものであるとし，pH が反応生起に大きな影響をおよぼすことから，恐らくは荷電関係による吸着ではないかと考え，Caruso (1938)[13], Savelli and Caruso (1938, 1939)[14], Danielli (1953)[12]らもアスコルビン酸の葉緑体への局在には疑問をもち，Höfler (1939)[15]もソラマメの孔辺細胞では，気孔が閉じた時には典型的な Molisch 反応が起るが，開いた時には一様に細胞内に分散していることを報告しており，細胞内特定構造への局在というより他に，何か重要な要因が介在しているだろうことが推測される．すでに永井 (1950)[8]が注意し前報[4])および本実験でも認められたように試薬の pH が反応の様相に大きく影響する．このことは条件によっては特定物質による特異的反応性が低下して，他にも反応にかかってくるような物質があるからということも考えられようが，クロマトグラムの顕色剤としての硝酸銀試薬は，酸性でも，中性でも，アンモニア性でも，本報の場合には一応は大差なく反応し，現われるスポットは皆同じ位置であり，*in vivo* でも一次的還元そのものは程度に多少の差はあっても，たいてい生じている．試薬の pH の如何によって，それぞれの場合の反応物質が違うとはこの場合考えられない．ただ，いわゆる Molisch 反応の生起…すなわち葉緑体への吸着と推測する…だけが，特定 pH 値その他の条件に支配されているわけである．

本実験でも，オオバチョウチンゴケでは部分による不一様が目立ったが，すでに Liebaldt (1938)[16]は *Mnium* について，Weber (1937)[17] は *Selaginella helvetica*, Mirimanoff (1940, 1943)[18] は *Mnium* および *Lithops* について同一組織でも部分によって葉緑体の黒化が不一様に起ることをみているが，これらが還元性物質含有量の部分による差異にもとずくとするのは早計であって，Caruso (1938)[13], Savelli and Caruso (1939)[14]は，反応生起には何か微妙な細胞内の生理条件があずかることを示唆し，永井 (1954)[8]は反応色調は吸着する元還銀粒子の大きさが種々の反応条件によって影響されるせいであるとしており，同一組織でも何か生理条件に差異があるせいと思われる．

飯島・平岡 (1950)[11]によれば，アスコルビン酸含有量が十分大である場合には pH 3 附近の試薬が最適であるが，含量が小であるものでは pH 5.4 程度の試薬を使用すべきこと，ただし，酸性以外ではアスコルビン酸に対する特異性が失われるから慎重を要することをモデル実験によって明らかにしているが，pH 3.2 で最も好適な，他よりも特にきわ立った Molisch 反応を示すナミガタタチゴケも，pH 5.6 が最適である他の種でも，ペーパークロマトグラムのスポットの位置，大きさ，強さから比較して物質的にはもちろん，その含有量にも大差があるとは思われない．

また細胞もしくは葉緑体の健全度と反応の度合とは，密接に連関していて，病的衰退に平行し，いちじるしく反応が弱まり，死んだものでは反応が負であることは従来よく知られ，尾形・永井 (1953, 1954)[19]は海藻類についても生死や傷害程度の判定にこの反応の度合を標準にとることが実用的にも有効な手段であることを明らかにした．沈水植物についての前報[4])の結果もこの点については全く一致していた．本実験の結果も大凡これに準ずることが多かったが，またそのような関係が不明瞭である場合もあり，さらには傷害の影響を蒙っていると思われる部分でかえっていちじるしい促進が起るという逆の例も顕著であった．しかし，そのような場合，決して傷害部周辺域において細胞内に還元性物質が量的に急増したとは思われず，これは傷害による刺戟が起した細胞内の微妙な生理的変化，例えば pH あるいは透過性の変動などが，そのような場合にはかえって反応生起に好適な促進的条件を与えているものと推測される．また，ナミガタタチゴケでは試薬の pH によって，傷害周辺域における反応の正負が逆転しており，反応負である場合にも，必ずしも還元性物質が失われてしまったわけではないことを示し，これらの事実は，反応の度合は直ちに還元性物質の量的消長関係を示すとする従来の考えとは必ずしも一致しない．

以上種々の結果は結局，硝酸銀反応の生起，いわゆる Molisch 反応は特に，すべての場合に直接に還元性物質の量的関係や局在性などにのみ平行するも

のとはいえないということを示唆するものであって，永井[20]も Molisch 反応とアスコルビン酸との一義的な関係は限定された植物材料において限定された実験条件の下にのみ成り立つもので，またアスコルビン酸だけが還元に対して責任を負うものでもないと考え，すでに (1950)[8] pH が支配的要因の一つであることを指摘しているが，本実験の結果はさらにまた，場合によってはより以上に，*in vivo* での反応の生起には，細胞自体の何か微妙な生理的内因が重要な関係をもっていることを推測せしめる．そのような内因が，具体的にはどのような要素によって支配されているかは，今論議する資料は得られていないが，それらが場合によって，すなわち被検種によって，組織，さらには細胞によってそれぞれ多少づつ特殊性があると考えることによって，ここに論じたいくつかの従来の知見とは相矛盾する特殊例を解明する手がかりとなるのではないだろうか．よく知られたように，死滅した細胞では反応能は速かに失われ，Weier (1938)[21]その他によればこれは酸化の結果還元力が失われてしまうからであるとされている．しかし Mirimanoff（永井 (1954)[8]の綜説より）によれば *Bryum capillare* では 0.1% Phenylmercuric borate で殺しアスコルビン酸を洗脱したものでも，なお正の反応が認められ，これは反応がアスコルビン酸の局在によるよりも葉緑体の表面特性によるところが大きいことを示すと報告している．一方，またクロマトグラフィーでは磨砕抽出液ですらメタ燐酸が安定剤として加えられれば，その操作中充分強い還元力が保持されているにもかかわらず，たとえメタ燐酸で浸潤処理をしても *in vivo* では傷害をうけ反応能を現わさない．これは物質としては還元性物質は安定化されていたとしても，死滅ないしは傷害細胞では，反応生起に重要なこれら微妙な内的生理条件が破壊されてしまっているからと推論することも可能であるかもしれない．

結局，以上の結果を要約すれば，これら蘚および羊歯類数種の細胞における硝酸銀還元反応がアスコルビン酸以外の物質が主体となって起され，反応の強さは必ずしも還元性物質の量的関係によってのみ決定されるものではなく，反応生起のためには細胞自体の何らか微妙な内的生理条件が関与していること，また還元性物質は細胞内に一様に分散しているものであって葉緑体の特異的黒化，即ち Molisch 反応は，むしろ種々の微妙な外的および内的条件によって支配される二次的吸着に基ずくものと推測され，細胞の生死や健全度との関係もそのような内的生理条件の変動として理解されるべきであり，永井[20]も細胞の次元における生理学の再検討ということをいっており，前報[4]に主張したと同様に今回もまた硝酸銀還元反応をアスコルビン酸の細胞化学的検出法として用いる場合には，すべての場合に絶対的にその存在および局在性を示すものといいきれず，場合によって反応結果の判定にはその信頼度に関してなおより以上に慎重な考慮が払われるべきであり，また反応生起の機構についても細胞内の微妙な内的生理要因が再検討されなければならないと考える．

終りに有益な御助言を下された大阪市立大学，永井進助教授，本学教育学部，相馬悌介教授，東京教育大学，植田利喜造助教授に謝意を表する．

要　　約

4種の蘚，2種の羊歯の葉の細胞について硝酸銀還元反応を検討した．

1. クロマトグラムは，反応が従来ほぼ定説とされたアスコルビン酸によってではなく，未確認の他の物質が主体となって起されることを示した．

2. 反応の強さと還元性物質の量および局在性や細胞の健全度との密接な関連などについての従来の定説とは一致しないいくつかの現象が見られた．

3. 結局，これらのことから硝酸銀還元反応はアスコルビン酸の細胞化学的検出法としてはすべての場合にその信頼性が確実とはいいきれず，結果の判定にはなおより以上に慎重な考慮を要すると思われる．

文　　献

1) Giroud, A., Leblond, C.P., Ratsimamanga, R., et Rabinowicz, M., Bull. d'Histol. appl. **11** : 369 (1934), Protoplasma **25** : 115 (1936). 2) Bourne, G., Anat. Rec. **66** : 369 (1936). 3) Molisch, H., Sitz. ber. kais. Akad. Wiss. Wien **1** : 127 (1918). 4) Yoshida, Y., Bot. Mag. Tokyo **71** : 57 (1958) in Jap. with Eng. Summary. 5) Dixon, M., and Webb, E.C., Enzymes,

London (1958). 6) Arnon, D.I., Science **122** : 9 (1955), Ann. Rev. Plant Phys. **7** : 325 (1956), Chloroplasts and Photosynthesis, Brookhaven Symp. in Biol. No. **11** : 181-235 (1958), Agrochimica **3** : 108 (1959). 7) Whatley, F.R., Allen, M.B., and Arnon, D.I., Biochim. et Biophys. Acta **32** : 32 (1959). 8) Nagai, S., J. Inst. Polytech. Osaka City Univ. D. **1** : 33 (1950), ibid. **2**:1 (1951), ——, and Ogata, E., ibid. **3** : 37 (1952), ——, ——, ibid. **3** : 46 (1952), ibid. **4** : 27 (1953), Protoplasma **54** : 444 (1954). 9) Mirimanoff, A., Rev. Cytol. Cytophys. Veget. **3** : 119 (1938). 10) Shinke, N., and Shigenaga, M., Rep. 74 Div. Sci. Council (1947) in Jap. 11) Iijima, M., and Hiraoka, T., Bot. Mag. Tokyo **63** : 278 (1950) in Jap. with Eng. Summary. 12) Danielli, J.F., Cytochemistry, New York (1953). 13) Caruso, C., Protoplasma **30** : 341, 481, **31** : 98, 489 (1938). 14) Savelli, R., and Caruso, C., ibid. **32** : 397 (1938), **32** : 517 (1939). 15) Höfler, R., ibid. **33** : 258 (1939). 16) Liebaldt, E., ibid. **31** : 267 (1938). 17) Weber, F., ibid. **28** : 283 (1937). 18) Mirimanoff, A., Bull. Soc. Bot. Genève **30** : 1 (1940), C. r. Soc. Sci. Phys. Hist Nat. Genève **60** : 105 (1943). 19) Ogata, E., and Nagai, S., Bull. Jap. Soc. Sci. Fisher. **19** : 1750 (1953), Physiol. Ecol. **6** : 10 (1954) in Jap. with Eng. Summary. 20) after the recent private communication (1959). 21) Weier, E., Amer. J. Bot. **25** : 501 (1938).

Summary

Several critical observations on the silver-nitrate-reduction in the cells of four species of musci and two species of ferns were carried out.

1. The trials to detect the reducing substance by means of chromatography in these materials showed that the reducing agent was not ascorbic acid but unknown other substance.

2. Observing the reactions which were carried out in this study, some contradictious facts which disaccorded with the present investigators' view that there were some close associations of the reaction-intensity with the quantity and the localization of the agent in cells and the intactness of the cell were noticed.

3. From these results, it must be more carefully considered that the silver-nitrate-reduction is not always sufficiently the specific reaction for ascorbic acid in the cytochemical use.

花粉の生理・形態学的研究

第 18 報 トウモロコシの花粉の窒素代謝について

沢　田　義　康*

Yoshiyasu SAWADA*: Physiological and Morphological Studies on the Pollen Grain Part 18. Nitrogen Metabolism of the Pollen Grain in *Zea Mays* L.

1959 年 11 月 24 日受付

前報[1]にて，*Paris hexaphylla* Chamisso.（クルマバックバネサウ）の花粉は sucrose agar 培地上で発芽困難であるが，その花粉，および雌ずい組織中に含まれるアミノ酸を，sucrose agar 培地に適量添加することにより，花粉の発芽および花粉管の伸長がいちじるしく促進されることを報告した．

本研究においては，sucrose agar 培地上で同様に発芽困難な *Zea Mays* L.（トウモロコシ）の花粉の発芽に対する，単一ならびに混合アミノ酸添加の影響を検するとともに，この花粉の呼吸におよぼすこれらアミノ酸の影響，さらに花芽生育の時期別にみたアミノ酸，および蛋白態窒素含量の消長，ならびにこの花粉中の transaminase 活性について報告する．

実験材料ならびに実験方法

（1） 供 試 材 料

Sucrose agar 培地上では，発芽が極めて困難な *Zea Mays* L. の一品種「ゴールデン・バンダム」を材料植物としてえらび，花器形成の初期より開花後にいたる各時期の雄ずい，雌ずいおよび苞を材料とした．

（2） 実 験 方 法

予備実験により，花粉の発芽培地としての最適 sucrose 濃度は 15%，最適 agar 濃度は 1 %，pH 6.5 であることを確かめた．よって本実験を通じこの条件で，sucrose agar 培地にそれぞれ 0.0005 %，0.001% および 0.002% アミノ酸を添加した．他方 aspartic acid, glutamic acid および cysteine の各アミノ酸と，他の各種アミノ酸との組合せによる 2 種アミノ酸混合添加培地をつくり，これに花粉を播種した．25° で 1 時間培養し，前報[1]の方法により，花粉の発芽率を検した．なお使用したアミノ酸は味の素株式会社製のもので，前報[1]と同種類のものを用いた．他方，花粉，柱頭および子房に含まれるアミノ酸の種類については，前報[1]に準じペーパー・クロマトグラフィにより検索した．つぎに花粉の呼吸の測定[2]は，sucrose 濃度 15%，各種アミノ酸は 0.0005%，0.001% および 0.002% として葯裂開直後の花粉 100 mg を材料として，Warburg 装置により 25° にて 1 時間の酸素消費量を測定し，呼吸量を μl にて示した．

つぎに花芽形成の初期より開花後にいたる時期別の，蛋白態窒素およびアミノ酸含量の変化については，micro-Kjeldahl 法，および Van Slyke 法により分析し，測定値は試料の生重量 1 g 中に含まれる含量を mg 量で示した．また花粉の transaminase[3,4,5,6] 活性の検定には，まず花粉 15 g に蒸溜水 20 cc を加え，乳鉢中でよく磨細し，3000 r.p.m. で 20 分間遠心分離し，上澄液を pH 7.5 の 0.1 M の燐酸塩緩衝液に 48 時間，3～5° で透析した後，これを酵素液として使用した．アミノ基の供与体としては，花粉発芽に供試した各種アミノ酸を用いた．アミノ基の受容体としては，pyruvic acid, α-ketoglutaric acid をいずれも苛性ソーダにて pH 7.5 に中和して使用した．アミノ酸溶液（0.05 M）α-ketoglutaric acid（0.05 M）0.5 ml，0.5 ml に酵素液 1.0 ml を加え，30° で 2 時間反応させた後，沸騰水中に 5 分間入れて反応を停止させた．ついで遠心分離を行ない，上澄液 0.001 ml を東洋沪紙 No. 50 の原点に spot としてつけ，一次元法の

* Division of Biology, Asahikawa Branch, Hokkaido Gakugei University, Hokkaido, Japan.
北海道学芸大学旭川分校生物学教室

ペーパー・クロマトグラフ法により展開させ，各アミノ酸とケト酸との間の transamination の有無を検した．

実験結果および考察

（1） 花粉の発芽におよぼす単一ならびに混合アミノ酸添加の影響

まず単一アミノ酸添加培地では，とくに 0.0005 %の aspartic acid, cysteine, glutamic acid, histidine, methionine および phenylalanine の添加によりいちじるしい発芽促進がみられた．しかし 0.0002 % アミノ酸添加培地では，却って発芽抑制の傾向がみられた(第1表参照)．よって単一アミノ酸添加培地で，顕著な発芽促進がみられた aspartic acid, glutamic acid, cysteine を中心として，他の各種アミノ酸と組合せた2種アミノ酸混合培地を作り，発芽状況を検して第2表に示す結果をえた．まず aspartic acidと各種アミノ酸との混合培地では，いずれも単一アミノ酸培地に比較して，いちじるしい発芽促進がみられたが，中でも alanine, arginine, cysteine, glycine および threonine との組合せの場合に顕著な促進効果がみられた．つぎに glutamic acid または cysteine と他のアミノ酸との二種混合培地においても，いちじるしい発芽促進がみられた．

第1表 花粉の発芽におよぼす単一アミノ酸の影響

アミノ酸の種類	アミノ酸濃度		
	0.0005%	0.001%	0.002%
Control	7.5%	7.5%	7.5%
Alanine	17.4	8.5	5.0
Arginine	trace	trace	trace
Aspartic acid	48.8	21.0	0
Cysteine	44.1	11.9	0
Cystine	13.1	trace	0
Glutamic acid	37.6	15.2	0
Glycine	8.2	trace	trace
Histidine	13.8	11.5	10.8
Hydroxyproline	10.7	trace	trace
Leucine	9.8	trace	trace
Lysine	10.0	trace	0
Methionine	30.0	trace	trace
Phenylalanine	20.6	6.6	0
Proline	9.1	9.1	9.0
Serine	23.0	9.1	8.3
Threonine	18.0	5.6	0
Tryptophan	trace	trace	trace
Tyrosine	trace	trace	trace
Valine	13.7	trace	trace

第2表 花粉の発芽におよぼす2種アミノ酸混合培地の影響

アミノ酸の種類	アミノ酸濃度		
	0.001%		
	Aspartic acidとの混合培地	Glutamic acidとの混合培地	Cysteineとの混合培地
Control	7.5%	7.5%	7.5%
Alanine	56.9	41.6	39.4
Arginine	54.0	38.3	54.4
Aspartic acid	21.0	53.8	33.5
Cysteine	53.8	11.9	11.9
Cystine	51.0	52.4	43.1
Glutamic acid	33.5	10.0	15.2
Glycine	68.0	47.8	54.4
Histidine	35.8	33.3	38.4
Hydroxyproline	37.6	47.1	32.0
Leucine	42.9	39.5	35.0
Lysine	52.0	37.6	24.0
Methionine	57.5	30.0	38.4
Phenylalanine	41.8	24.3	50.5
Proline	32.5	49.6	42.6
Serine	50.9	61.8	45.8
Threonine	66.5	47.6	60.9
Tryptophan	32.9	63.0	27.6
Tyrosine	35.2	33.6	29.4
Valine	41.0	51.5	45.4

以上からも明らかであるごとく，sucrose agar 培地上における *Zea Mays* L. の花粉の発芽は，適濃度の単一アミノ酸添加により促進されるが，さらに適当な組合せの二種アミノ酸の混合添加により発芽促進効果は強調される．久保[7]は，ゼラチン培地上で，*Zea Mays* L. の花粉の高率の発芽がみられたと報告したが，恐らくゼラチンの加水分解によって生ずるある種のアミノ酸の混合的な作用がこれに関与するものと推察される．

（2） 花粉の呼吸におよぼすアミノ酸の影響

花粉の呼吸におよぼす 0.0005% の各種アミノ酸の影響については，第3表に示すごとく，arginine, glutamic acid, leucine, methionine, phenylalanine

第3表　花粉の呼吸におよぼすアミノ酸の影響

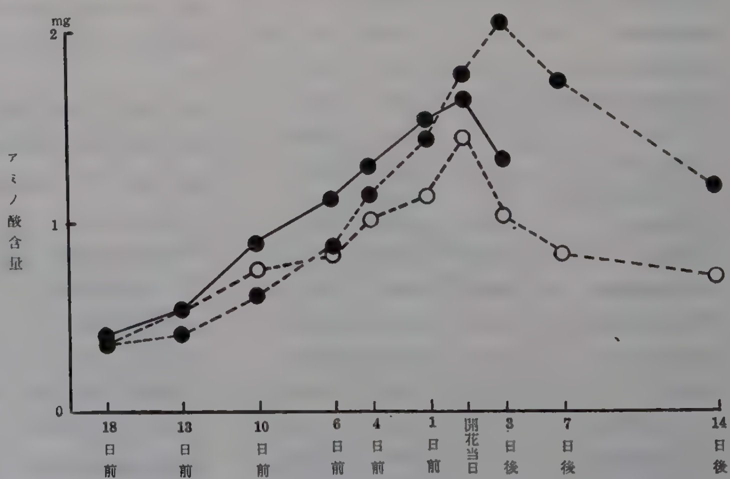
アミノ酸の種類	アミノ酸濃度		
	0.0005%	0.001%	0.002%
	$\mu l O_2$	$\mu l O_2$	$\mu l O_2$
Control	1349.00	—	—
Alanine	1393.14	1481.03	846.30
Arginine	2105.00	1307.00	894.73
Aspartic acid	1438.50	1393.80	1171.80
Cysteine	1360.13	1176.30	880.00
Cystine	1359.76	1387.00	1329.82
Glutamic acid	1997.29	1228.20	1156.00
Glycine	1523.61	1472.86	1153.14
Histidine	1539.00	1246.70	987.35
Hydroxyproline	1323.19	1088.56	917.90
Leucine	1614.48	1410.00	876.86
Lysine	1550.24	1202.85	1192.78
Methionine	1643.90	1142.49	973.71
Phenylalanine	1749.00	1341.62	897.00
Proline	1622.50	1100.00	962.28
Serine	1718.75	1270.15	863.10
Threonine	1636.18	1341.60	1235.10
Tryptophan	1605.62	1388.75	1304.72
Tyrosine	1501.25	1213.03	1199.76
Valine	1423.13	1109.60	1185.98

および threonine の各アミノ酸の添加により，呼吸はいちじるしく増加した．さらにアミノ酸濃度を 0.001% に高めると，呼吸はやや減少したが，しかし control に比すると，わずかながら花粉の呼吸は促進された．さらにアミノ酸濃度を増加した 0.002% では，いずれのアミノ酸添加の場合も花粉の呼吸は急激に減退した．このことは濃度の高いアミノ酸の添加により，花粉の発芽も抑制されることと，何らかの関係があるごとく思われる．他方，低濃度のアミノ酸の添加により花粉の呼吸増加をみた種類のアミノ酸は，また同時に，その添加により花粉の発芽はいちじるしく促進される．このことは，sucrose agar 培地にこの種類のアミノ酸を添加することにより，ある種の呼吸系を通じて物質代謝を促進し，これが花粉の発芽のエネルギー源となり，花粉の発芽促進を結果するものと考えられる．

（3）雄ずいおよび雌ずいに含まれるアミノ酸の種類，ならびに含量と，transaminase 活性

Zea Mays L. の開花 18 日前の時期より，開花後にいたる時期別の花器内アミノ酸含量の変化は第1図の如くである．すなわち開花 10 日前頃より，雄ずいの形成進展にともなって，顕著なアミノ酸含量の増加がみられ，開花当日にアミノ酸の蓄積も最大量に達し，ここに雄ずいの充実をみるが，しかし，葯裂開後は急激に減少した．つぎに雌ずいのアミノ酸含量の変化についてみると，開花6日前より子房中に急激な増量がみられ，開花3日後に最大量を示した．また苞についてみると，花芽形成の進展にともない，次第にアミノ酸含量の増加がみられ，開花当日を最高として，以後減退した．

つぎに，このようなアミノ酸含量の消長を示した雄ずい，および雌ずいの開花当日におけるアミノ酸の種類についてみると，いずれの組織にも alanine, arginine, glutamic acid, leucine, phenylalanine および serine の各種が多量に含有される．しかもこの場合花粉，および雌ずいに共通したアミノ酸が含有される．



第1図　開花前後における雄ずい，および雌ずい内のアミノ酸含量
●——● 葯，●……● 子房，○……○ 苞．

第4表　花粉，および雌ずいに含まれる遊離アミノ酸

アミノ酸の種類	花　粉	柱　頭	子　房
Alanine	卌	╫	卌
Arginine	╫	+	╫
Aspartic acid	+	+	+
Cysteine	−	−	−
Cystine	−	−	−
Glutamic acid	卌	╫	╫
Glycine	+	−	−
Histidine	−	−	−
Hydroxyproline	+	+	+
Leucine	+	+	卌
Lysine	+	+	+
Methionine	−	±	+
Phenylalanine	+	+	卌
Proline	+	+	+
Serine	╫	╫	+
Threonine	╫	+	╫
Tryptophan	−	−	+
Tyrosine	+	+	+
Valine	+	+	卌
Unidentified	+	+	+

Sarkar[8] らは *Zea Mays* L. の花粉中には遊離のアミノ酸と，蛋白の加水分解によるものをあわせ 12 種のアミノ酸を報告したが，本実験においては，遊離アミノ酸として 14 種と，不詳の種類1種がみられた（第4表参照）．しかもこれらの中で花粉，柱頭，子房に共通して多量含有される種類のアミノ酸は，いずれも花粉の発芽を促進し，かつ花粉の呼吸を増加させることは興味がふかい．なお花芽の形成進展に伴なうアミノ酸の種類の時期別による消長を明らかにするため，開花 18 日前の雄ずいと開花当日の雄ずいについて分析したところ，全く同一種類のアミノ酸が検出された．このことは，アミノ酸含量の消長は，種類の増減によらず，ある限られた種類のアミノ酸の量的変化によるものであることを示す．また開花期に雄ずい中に含まれる蛋白態窒素を加水分解して構成アミノ酸の種類を検討したところ，遊離アミノ酸と同一種のものがみとめられた．

つぎに花粉に含まれるアミノ酸相互間における transamination について検討し，第5，6表に示す結果をえた．これよりみると glutamic acid と aspartic acid および glutamic acid と alanine と

第5表　各種アミノ酸と α-ketoglutaric acid とより glutamic acid の生成

アミノ酸の種類	生成 glutamic acid の spot の比較値
Alanine	卌
Arginine	±
Aspartic acid	╫
Cysteine	−
Cystine	±
Glycine	±
Histidine	−
Hydroxyproline	−
Leucine	±
Lysine	±
Methionine	±
Phenylalanine	±
Proline	−
Serine	±
Threonine	−
Tryptophan	±
Tyrosine	±
Valine	±
Control	−

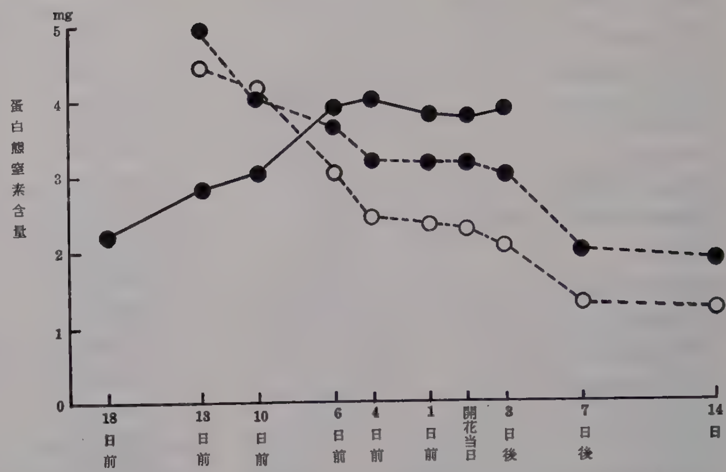
±の記号は glutamic acid の spot がかすかにみとめられることを示す．

第6表　各種アミノ酸と pyruvic acid より alanine の生成

アミノ酸の種類	生成 alanine の spot の比較値
Arginine	−
Aspartic acid	±
Cysteine	−
Cystine	±
Glutamic acid	卌
Glycine	±
Histidine	−
Hydroxyproline	−
Leucine	±
Lysine	±
Methionine	±
Phenylalanine	±
Proline	±
Serine	±
Threonine	±
Tryptophan	±
Tyrosine	±
Valine	±
Control	−

±の記号は alanine の spot がかすかに認められることを示す．

の間の transaminase の活力が最も高く，その他のアミノ酸と pyruvic acid，または α-ketoglutaric acid との transaminase の活力も弱いながらみとめられた. Albaum および Cohen[9]によると発芽燕麦中における glutamic acid と aspartic acid との transaminase 活性は，発芽の初期に強く，ついで，めばえでの全蛋白含量の増大が起ることを報告している．つぎに述べる花芽内における蛋白態窒素含量の消長にも，同様にこの transaminase 活性が関係することが考えられる．



第2図　開花前後における雄ずい，および雌ずい内の蛋白態窒素含量
●——● 葯，●……● 子房，○……○ 苞

（4）　雄ずいおよび雌ずいに含まれる蛋白態窒素含量

花芽の形成ならびに開花にともなう蛋白態窒素含量の変化について分析し，第2図に示す結果をえた．まず雄ずいについてみると，花芽形成の進展に伴ない急激な蛋白態窒素の蓄積がおこり，開花時に最高値がみられた．このことは，Howlett[10]がりんごについて，花芽の時期から花瓣落下の時期までの全窒素量を測定し，花芽時に比し，満開時には3倍の蓄積がみられたと報告している．本実験における花粉内のアミノ酸含量の増量と相まって，他の組織より可溶態の形で花粉内に転流してきた窒素が，蛋白態窒素の型で貯蔵され，花粉の充実，さらには花粉発芽の準備を整えるものと考えられる．つぎに子房，苞についてみると，これら両器官の生育に伴ない，蛋白態窒素含量はかえって急激に減退する．このことは開花期に花芽内アミノ酸含量が急増する事実を考慮すると，蛋白態窒素は葯，子房内で可溶態窒素の形となり花粉の発芽，伸長に用いられることが考えられる．

要　　約

（1） Sucrose agar 培地上で発芽が困難な *Zea Mays* L. の花粉は，培地に適当なアミノ酸を添加することにより，発芽がいちじるしく促進される．さらに適当な組合せの2種のアミノ酸を混合添加した培地では，発芽はさらにいちじるしく促進される．

（2） 花粉の発芽を促進する種類のアミノ酸は，また花粉の呼吸を増大させる．このことは，これらアミノ酸の添加により，花粉内の物質代謝が高まり，ひいては花粉の発芽を促進するものと考えられる．

（3） 上記のような特質をもつアミノ酸は，開花当日ないし開花後の雄ずい，ならびに雌ずいに集中的に蓄積する．かつこのような種類のアミノ酸は，雄ずい，および雌ずいに共通して含有され，しかも，花芽形成の時期別にみても，このようなアミノ酸の種類には変化がみられず，ただ量的消長があることを確かめた．

（4） *Zea Mays* L. の花粉内に transaminase 活性のあることを確認した．glutamic acid と aspartic acid および glutamic acid と alanine との間の transaminase の活力が最も顕著であった．

本研究にさいして，いろいろと御指導をいただいた，北海道大学農学部田川隆教授に深謝の意を表する．また実験にさいしては，味の素株式会社食品研究室戸井文一博士より種々御援助をいただいた．記して深く謝意を表わす．

文　　献

1) 沢田義康，植雑，**71** : 218 (1958). 2) Hellmers, H., and Machlis, L., Plant Physiol. **31** : 284 (1956). 3) 村上　浩・林　武，日農化，**31** : 468 (1957). 4) Cammarata, P.S., and Cohen, P.P., J. Biol. Chem. **187** : 439 (1950). 5) Green, D.E., Leloir, L.F., and Necita, V., ibid. **161** : 559 (1950). 6) 常岡健二・原田　尚，最新医学，**13** : 191 (1958). 7) 久保　淳，植雑，**71** : 282 (1958). 8) Sarkar, B.C.R., Witter, S.H., Luecke, R.W., and Sell, H.M., Arch. Biol. Chem. **22** : 353 (1949). 9) Albaum, H.G., and Cohen, P.P., J. Biol. Chem. **149** : 19 (1943). 10) Howlett, F.S., Cornell University, Agricult. Exptl. Station, Memoir. **99** (1926).

Summary

1. The pollen grain of *Zea Mays* L. germinates poorly on sucrose agar medium. By the addition of some kinds of amino acids to the culture medium either independently or by mixing with favourable combinations, however, the pollen germination was much enhanced.

2. Addition of the amino acids which accelerated the pollen germination caused the increase of the pollen respiration as compared with that of the control.

3. The amino acids which increased the germination and the respiration of the pollen grain, were found to be common to both the pollen grain and the pistil of this plant.

4. Enzyme transaminase was ascertained to be present in the pollen grain. In particular, the transamination reactions between glutamic acid and aspartic acid, and between glutamic acid and alanine were found to be active.

5. The amino acid content in the male and female flowers increased gradually as the flower bud matured. The maximum content was observed immediately after the time of anther dehiscence and thereafter the content decreased gradually.

Micrococcus glutamicus の細胞学的研究
第3報　極顆粒とリン酸含量の関係および有機酸酸化能について

板　垣　史　郎*

Shiro ITAGAKI*: Cytological Studies on *Micrococcus glutamicus*. Part III. On the Relationship between the Polar Granules and Phosphate Content, and Oxidative Activity of the Polar Granule on Several Organic Acids.

1959 年 12 月 3 日受付

緒　　言

Micrococcus glutamicus の形態的諸性状についてはすでにのべた[1,2]. その際，ある培養条件のもとでは，極在性の顆粒が明瞭に形成され，しかも，この顆粒は，いわゆる metachromasy をしめし，菌体構成上特異なものであろうことを指摘した.

すでにこのような菌体内顆粒については相当多くの菌種について知られている．とくに著明なものとして，*Corynebacterium diphtheriae* について，Ernst[3]，Neisser[4] および Babes[5] らが早くから認めている．その他にも Meyer[6] は Yeast 中にも同様な顆粒をみとめた．このような顆粒は，metachromasy を示すことから，metachromatic granule (以下 m. granule) とよばれているが Babes-Ernst granule, volutingranule などともいわれる．その後，*Aspergillus*[7]，*Mycobacterium*[8]，*Aerobacter*[9]，*Cloaca cloacae*[10]，*Salmonella paratyphi* B[11] および *Micrococcus lysodeikticus* などをはじめ，多くの菌についても知られるようになった．著者も数種の *Corynebacterium*, *Brevibacterium* および *Micrococcus* などにおいて認めている．この顆粒の本態については，はじめ Piekarski[12] や Knaysi および Mudd[13] は，核あるいは核酸と考え，また Mudd[14] はその後 mitochondria であると主張した.

* 協和醱酵工業株式会社東京研究所　Tokyo Research Laboratory, The Kyowa Fermentation Industry Co. Ltd., Tokyo, Japan.

一方，この顆粒は，酢酸鉛染色による phosphate granule と一致し[15]，また培養条件によって無機直鎖のポリリン酸量と顆粒の発現が平行関係を示すことが Wiame, Lefebvre[16,17] および Mudd[18,19] らによって明らかにされた．さらに電子顕微鏡的にも同一菌体について electron dense granule と metachromatic granule が同一のものであることが証明されている[20,21].

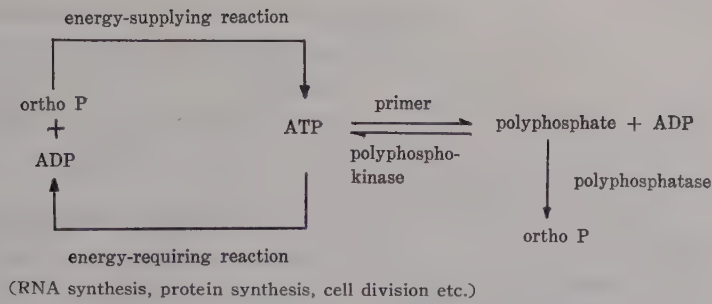
このような事実を総合して，今日では metachromatic granule は，ポリリン酸を主成分とすることは，ほとんど確実といってよいであろう.

以上のごとき文献的考察と，*M. glutamicus* の極顆粒に関する既報のごとき観察より，本菌の顆粒もポリリン酸顆粒であろうと想像される．菌体中の，このような無機のポリリン酸が，一体いかなる生理的意義をもつものであるか，ということについては，未だ完全に明白になっているとはいい難いが，吉田氏[22]は第1図および第2図に示すごとく，リン酸代謝経路と菌体分裂の関係をしめしている.

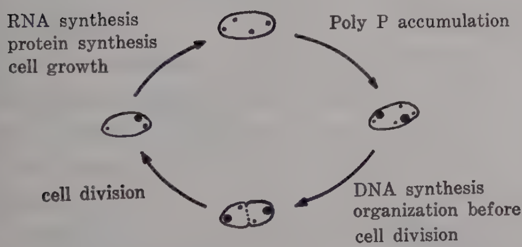
すなわち，菌体内で，ATP, ADP, polyphosphate および orthophosphate は，動的平衡にあり，energy-supplying reaction によって，ortho-P は，ADP と結合して ATP となり，逆に energy-requiring reaction ではこの反対の左の方へ平衡点が移動する.

このように poly-P は ADP にそのリン酸基を移すことによって energy 供給源となり，RNA などのリン酸供給源となる.

かくの如く，リン酸の菌体内における意義はきわめて大きいことより，本 *M. glutamicus* において，



第1図　菌体内のリン酸代謝経路（吉田[22]）



第2図　菌体の生長とポリリン酸の消長（吉田[22]）

m. granule の発現とポリリン酸量の関係，m. granule をもつ菌体と，しからざる菌体とのリン酸量の比較，および種々の enzymatic activity との関係を追求する目的で実験をおこなった．

実験と結果

使用菌株 *Micrococcus glutamicus* 541

1. Metachromatic granule 形成培地の決定

合成培地において培養初期に m. granule の発現を認めたが，さらに培養を進めると，菌体が充実し，不整桿菌形態を呈する時期に一致して消失し，染色，電顕共に認められなくなる．ここにおいて m. granule 研究の第一歩として菌体に多数の m. granule を形成させる培養条件を決定しようとした．

実験方法

培地は前培養のために glucose bouillon，本培養には第1報[1])に示した合成培地を基本組成とした．

各培地成分，植菌量，pH などを種々変え，培養を経時的に観察し m. granule 形成の有無多寡を検討した．

結　果

精細な記述は省略するが，一般に合成培地では m. granule の形成は不安定な結果を示し，再現性にとぼしい．しかし，KH_2PO_4 および K_2HPO_4 をおのおの 0.5～1.0％程度に増加した場合，菌形は，肥大不整桿菌形態を示し，多細胞菌体となり，好塩基性が強まるにもかかわらず，m. granule の形成は明瞭である．

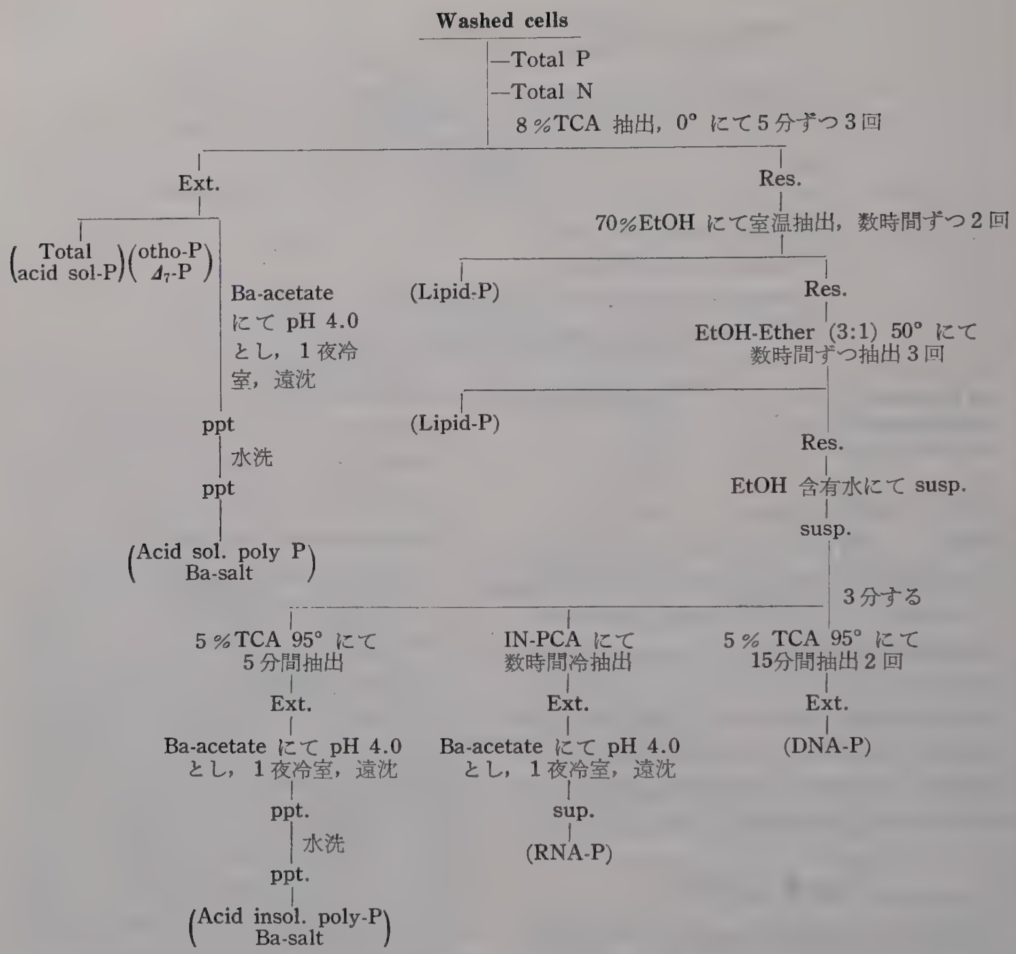
一方 *M. glutamicus* 534 においては，glucose bouillon では既報[1])のごとく m. granule の形成は比較的まれであったが，541 株では glucose bouillon においてもかなり良好な m. granule 形成をしめす．しかし，これにさらに KH_2PO_4 および K_2HPO_4 を各 0.25％ずつ添加することにより形成は，いちじるしく安定になり，多数しかもかなり大きな m. granule を形成する．このことより，リン酸添加培地を P-GPM 培地〔添加しない培地を GPM (glucose, peptone, meat-ext. の略) 培地と称する〕と呼称し，後の実験に用いることにした．

2. 菌体のリン酸量について

GPM および P-GPM 培地に培養した菌体内のリン酸化合物の量を分割定量し比較した．この結果，P-GPM 培地で培養した m. granule 形成菌体には acid insoluble polyphosphate が非常に多いことがわかった．

実験法

GPM および P-GPM 培地に2日間 28° に培養した *M. glutamicus* 541 の菌体を遠沈集菌し，冷生理食塩水にて3回洗滌後リン酸化合物の分割定量をおこなった．分割方法は Mudd, Yoshida および Koike[19]) の方法に準じた．第3図にその大要を示した．無機リン酸は高橋法[23])，その他のリン酸は Allen 法[24])，DNA-P は diphenylamine[25]) 反応，RNA-P は Orcinol[26]) 反応により定量した．また窒



第3図 リン酸化合物の分割定量

第1表 リン酸量の比較（表中の値は，すべて菌体 N 1 mg 中に含まれるリン酸化合物の含量を P の γ 数で示した）

区分 / 培地	全 P	酸可溶性 P			リピドP	酸不溶性 P		
		オルトP	acid labile P			RNA P	DNA P	ポリP
			ポリP	それ以外				
GPM	320.0	19.4	trace	55.7	21.4	115.1	17.4	0.6
P-GPM	251.7	15.0	trace	40.6	22.0	96.8	13.0	18.2

素の分析は滴定法[27])によった．

結　果

それぞれの分割処理操作についてあらかじめ充分検討を加えた上で分割定量を行なった結果を第1表に示した．これによると acid insoluble poly-P を除いた各リン酸量は，GPM および P-GPM 各培地に培養した菌体間に大きな差はみられないが，後者は acid insoluble poly-P 量が圧倒的に多い（約 30 倍）ことが知られた．これよりしても m. granule が無機ポリリン酸を主成分とするものであろうことが推定される．

3. 有機酸酸化能の比較

GPM および P-GPM で培養した各菌体間にどの程度酵素活性に差がみられるか，つまり m. granule の有機酸々化におよぼす影響を知る目的で数種の有機酸を用いて実験を行なった.

実験方法

培養: GPM 培地にて 24 時間培養した前培養を10%の割合で，GPM および P-GPM 培地に植菌し(それぞれ 2 *l* のフラスコに 500 m*l* の培地を含む) 28° にて振盪培養し，経日的に pH，菌体量を測定し methylene blue 染色標本により m. granule の観察を行なった.

ワールブルグ検圧法: 経日的に菌体をとり冷生理食塩水にて3回洗滌し tris buffer (pH 7.0) にて 70～100 mg/m*l* の濃厚な菌液を調製し，28° にて starvation を行ない，後 tris buffer にて 20 mg/m*l* の菌液とした.

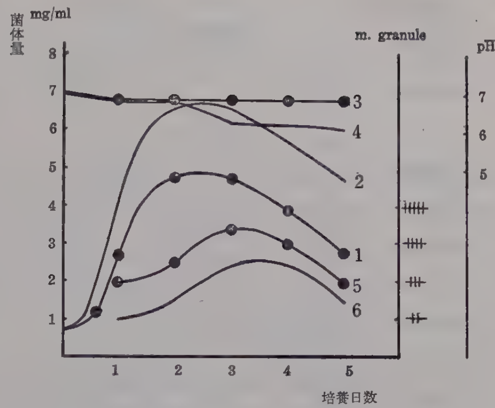
各基質有機酸は Na-salt の形で用い，100 μM/m*l* の濃度になるように調製した.

ワーブルグ容器にはつぎのように添加した.

Main:	基質 (100 μM/m*l*)	1.0 m*l*
	M/15 Sodium phosphate buffer (pH 7.0)	1.0 m*l*
	M/15 KCl	1.0
	tris buffer (pH 7.0)	1.0
	deionized water	0.3
Center well:	20%KOH	0.2
Side arm:	cell susp. (20 mg/m*l*)	0.5

結　果

pH,細胞生長および m. granule の形成: (第2表) および (第4図) に明らかなごとく P-GPM では生長がややおとるが m. granule の形成は GPM より良好である. P-GPM において生長がおとることは，第1図および第2図より明らかなごとく，リン酸量が多量に存在するため，平衡点は energy-supplying reaction の方へ傾くため，細胞分裂 (energy-requiring reaction) がややよくせいされるためと解釈できる.



第4図 菌体量，pH，metachromatic granule の変動
1, 2: 生育，3, 4: pH，5, 6: m. granule
—— GPM 培地，—●— 培地を示す.

有機酸酸化能について: 培養経日的菌体の有する有機酸酸化能を一括して Q_{O_2} 値として第3表に示した.

これによると新しい培養程活性が強く，漸次弱まる傾向にあるといえよう. この間の関係をさらに明瞭にするために第5図，A および B を載せた. 要約すると

最も強く酸化を受ける基質‥‥Acetate, Lactate

中等度‥‥Pyruvate

弱いもの‥‥L-Malate, Succinate, Fumarate. Formate, Citrate

また GPM, P-GPM において根本的な酵素活性の差は認められない.

第2表 菌体量，pH，metachromatic granule の変動の比較

Incub. Day	P-GPM 菌体量 mg/m*l*	P-GPM pH	P-GPM m. granule	GPM 菌体量 mg/m*l*	GPM pH	GPM m. granule
1	2.8	6.9	卅	4.2	6.8	卄
2	4.8	6.8	卅±	6.6	6.8	卄±
3	4.7	6.8	卌±	6.6	6.2	卅±
4	3.9	6.8	卌	5.7	6.2	卅±
5	2.7	6.8	卅	4.7	6.0	卄±

第3表　経日的 Q_{O_2} 値の変化（菌体, 10 mg, 2時間測定値より換算）

Substrate	incub. day med.	1	2	3	4
Fumarate	GPM	14.5	8.5	6.2	3.3
	P-GPM	12.0	—	3.1	1.3
L-Malate	GPM	17.0	5.7	4.1	1.3
	P-GPM	8.8	—	4.7	0.9
Pyruvate	GPM	—	25.5	27.2	25.0
	P-GPM	24.5	24.0	22.5	21.7
Lactate	GPM	30.0	49.0	39.0	38.5
	P-GPM	44.5	35.5	32.5	35.0
Formate	GPM	12.7	10.7	8.5	4.7
	P-GPM	10.7	9.7	3.7	1.5
Succinate	GPM	16.6	—	7.8	4.3
	P-GPM	13.3	10.7	5.9	1.2
Citrate	GPM	2.0	1.4	1.1	0.7
	P-GPM	2.6	1.3	—	—
Acetate	GPM	40.7	35.5	—	27.7
	P-GPM	53.2	46.0	34.5	35·2

考　　察

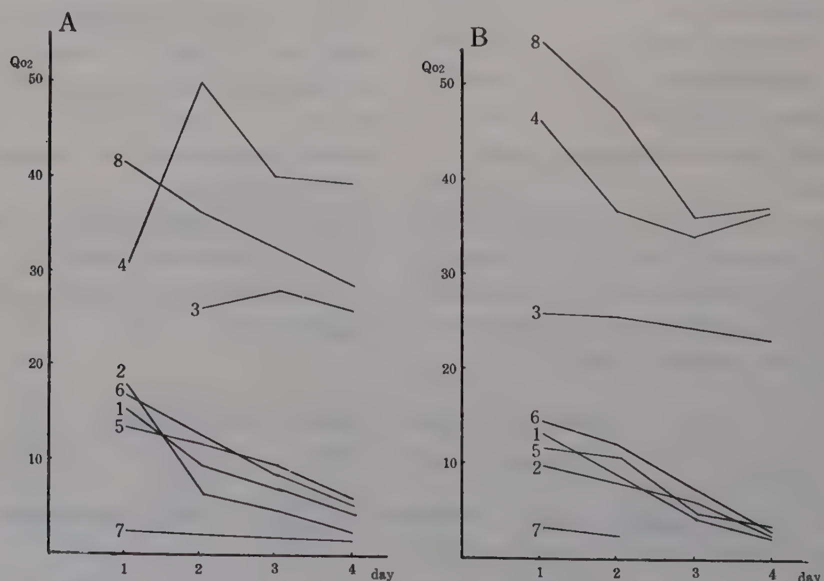
1. Metachromatic granule 形成培地の決定

M. glutamicus 534 は, glucose bouillon 培地ではほとんど m. granule を形成せず, biotin を添加した合成培地においてのみ良く形成する. しかも, この m. granule は培養の初期にのみ認められ, 菌体が肥大, 充実するにつれて認められなくなることはすでに述べたが, *M. glutamicus* 数株のうち, 本実験に供した 541 株は最も m. granule を形成しがたく, 合成培地では, ほとんど形成しないにもかかわらず, glucose bouillon 中に無機リン酸を添加したところ, 推測のようにきわめて良い結果を得た. 合成培地ではなく glucose bouillon でよく形成するということの原因については明かでないが, 結局菌の生理の問題であり, このような点についてはさらに検討したい.

2. 菌体のリン酸量について

第1表に示したように, m. granule を多数有する菌体は, acid insol. poly-P が多量に存在する. このことは, m. granule は, オルトリン酸が, 直鎖に縮合したポリリン酸よりなることをしさする.

菌体内のポリリン酸は, 培地中のリン酸からよう



第5図　有機酸々化能の経日的変化: Q_{O_2} 値による表示.
A: GPM 培地生育菌体　　B: P-GPM 培地生育菌体
1. fumarate　2. L-malate　3. pyruvate　4. lactate
5. formate　6. succinate　7. citrate　8. acetate

いにつくられることは P^{32} を用いた実験により明らかにされている[28]．本実験に示したように，リン酸添加培地においてよく m. granule を形成し，しかしそのような菌体中には，いちじるしく，ポリリン酸が蓄積されていることよりしても，本菌の m. granule もリン酸顆粒であることは間違いないところであろう．

3. 有機酸酸化能について

前述したように，菌体内のリン酸というものは，菌の生理・代謝に非常に重要な役割をもっているものである．したがって，このリン酸を蓄積した菌体では当然いろいろの酵素活性に差ができてくるものと予想される．そこで，種々の有機酸を基質として，これらの有機酸を酸化する力にいちじるしい差を生ずるかどうかを調べてみた．しかし，結果は第3表および第5図に示したように大きな差異をみとめることはできなかった．もちろん検討を加えた基質は，ごくかぎられたものだけであり，これのみでうんぬんするのは早計であるが，顆粒を形成しているポリリン酸は，少なくとも TCA cycle に対しては，大きい影響を与えないものと考えられる．

本菌は Lactate, Acetate に対する酸化能が特に強いが，Lactate 酸化能についてみると，GPM 培養菌体では，2日目が特に強くなり，その後やや弱まるに反し，P-GPM 培養菌体では1日目が最も強く，以後弱まるが，4日目にまたやや強くなる（Acetate 酸化能も同様な傾向にある）点が異る．その他に関してはほとんど一致している．

要　　約

1. *M. glutamicus* において，metachromatic granule を形成させる培地を検討し，glucose bouillon に，KH_2PO_4 および K_2HPO_4 を添加した P-GPM 培地を決定した．

2. P-GPM 培地にて培養され，いちじるしく m. granule を形成した菌体は，acid insoluble polyphosphate を多量に含む．その含量は単なる glucose bouillon 培地にて培養された菌体の含量の約 30 倍にもおよぶ．

3. *M. glutamicus* は，Acetate, Lactate を強く酸化する．このような有機酸酸化能は metachromatic granule が存在するか否かには無関係である．

有機酸酸化能は日を追って漸減する傾向にある．

種々御指導を頂いた東大教授湯浅明博士，いろいろと御教示をいただいた東大助教授吉田博士に深く感謝する．また御鞭韃をいただいた当社研究所長木下祝郎博士，および実験に協力をいただいた所員古川稔，森田晧暉両氏に深謝する．

文　　献

1) 板垣史郎・木下祝郎，植雑 **72** : 52 (1959). 2) ——, ——, 同 **72** : 114 (1959). 3) Ernst, P., Z. Hyg. **4** : 24 (1888). 4) Neisser, A., ibid. **4** : 165 (1888). 5) Babes, U., ibid. **5** : 173 (1889). 6) Meyer, A., Bot. Zentr. **62** : 113 (1904). 7) Mann, Y., Biochem. J. **38** : 339, 345 (1844). 8) Mudd, S., Winterscheid, L. C., DeLamater, E. D., and Henderson, H. J., J. Bact. **62** : 45 (1951). 9) Smith, I. W., Wilkinson, J. E., and Duguid, J. P., ibid. **68** : 450 (1945). 10) 武谷健二，第 30 回細菌学会総会講演 (1957). 11) ——, 医学のあゆみ **24** : 325 (1957). 12) Piekarski, G., Z. Bakt. Parasitenk. Ab, Ib, Org. **144** : 140 (1939). 13) Knaysi, G., and Mudd, S., J. Bact. **45** : 349 (1943). 14) Mudd, S., Ann. Rev. Microbiol. **8** : 1 (1954). 15) ——, and Winterscheid, L.C., Exp. cell Res. **5** : 25 (1953). 16) Wiame, J.H., Biochem. Biophys. Acta **1** : 234 (1947). 17) ——, and Lefebvre, P.H., Comp. rend Soc. Biol. **140** : 921 (1946). 18) Sall, J., Mudd. S., and Davis, J.C., Arch. Biochem. Biophys. **60** : 131 (1956). 19) Mudd, S., Yoshida, A., and Koike, K., J. Bact. **75** : 224 (1958). 20) Winkler, A., Symp. 6th Congr. Int. Microbiol. (1953). 21) Glauert, A.M., and Breeger, E.M., J. Gen. Microbiol. **13** : 310 (1955). 22) 吉田昭，蛋白質核酸酵素 **4** : 39 (1959). 高橋泰常，生化学 **26** : 690 (1955). 24) Allen, R. J. L., Biochem. J. **34** : 858 (1940). 25) Dische, Z., The Nucleic Acids. Vol. I. 287, Academic Press (1955). 26) Mejbaum, W., Z. Physiol. Chem. **58** : 117 (1939). 27) 八木康夫，核酸および蛋白質上巻 p. 156 共立出版社 (1951). 28) Wiame, J.M., J. Biol. Chem. **178** : 919 (1949).

Summary

1. *Micrococcus glutamicus* strain 541 formed many metachromatic granules when it grew in glucose-bouillon medium with KH_2PO_4 and K_2HPO_4 (P-GPM medium).

2. Cells which formed many metachromatic granules during their growth in P-GPM medium contained considerable amounts of acid-insoluble polyphosphate. The content of such polyphosphate in the above mentioned cells was about 30 times higher than that of the cells which grew in glucose-bouillon medium without KH_2PO_4 and K_2HPO_4(GPM medium).

3. Oxidation of several organic acids by *M. glutamicus* was studied.

Among these organic acids, acetate and lactate were strongly oxidized by the organism. It seems that there is no relation between the oxidation activity on these organic acids and the existence of metachromatic granules in the cells.

The oxidation activity of *M. glutamicus* on these organic acids decreased gradually after the first day of the culture.

雑　　録

日本植物学集報について

日本学術会議編集の日本植物学集報 (Jap. Journ. Bot.) 第 17 巻，第2号が刊行され，次の8論文が登載されています。

1. Nakajima, G. Cytogenetical studies on the intergeneric F_1 hybrids between *Triticum macha* and four species of *Secale*.
2. Sharma, A. K., and Bhattacharyya, N. K. An investigation on the scope of a number of pre-treatment chemicals for chromosome studies in different groups of plants.
3. Sutô, T., and Sugiyama, S. Sex expression and determination in spinach. I. Growth habit and its sex-limited inheritance.
4. Tsuchiya, T. Cytogenetic studies of trisomics in barley.
5. Hotta, Y. The role of protein and ribonucleic acid in the differentiation of fern gametophyte.
6. Shibata, M., and Ishikura, N. Paper chromatographic survey of anthocyanin in tulip-flowers, I.
7. Tazaki, T. On the growth of pine yearings in coastal dune regions with special reference to their drought resistance.
8. Hogetsu, K., Oshima, Y., Midorikawa, B., Tezuka, Y., Sakamoto, M., Mototani, I., and Kimura, M. Growth analytical studies on the artificial communities of *Helianthus tuberosus* with different densities.

なお第 18 巻，第1号の原稿しめきりは，きたる 8月 15 日です。原稿は日本植物学集報編集委員あてに書留郵便でお送りください。原稿の体裁は最新号を参照してください。なお，原則として校正では文章を訂正することはできません。

チョウセンレンギョウの花のフラボノイド (植物色素 第X報)

山田節子*・高野俊武**・涼野 元**・林 孝三*

S. YAMADA, T. TAKANO, G. Suzushino, and K. Hayashi: Plant Pigment, X. Rutin as a Flavonoid Component in the Perianth of *Forsythia koreana*.

1959 年 10 月 14 日受付

チョウセンレンギョウ *Forsythia koreana* Nakai (レンギョウ科) はわが国の各地で栽植されている観賞用の低木で，春，葉に先立って開花し，鮮黄色の小花は全株を埋めて人目を引く．この花には，塩化第二鉄で暗緑色を呈する単一のフラボン体が含まれており，調査の結果それはルチン Rutin (Quercetin 3-rhamnoglucoside) と同定された．ただし，この花の黄色はカロチノイド色素によるもので[1]，ルチンは直接の要因ではない．

さきに，R. Kuhn ら[1] および Moewus[2] は *Forsythia intermedia* の 2 変種 var. *spectabilis* および var. *densiflora* の花粉からそれぞれ Rutin と Quercitrin とを分離し，これらの植物の自家不和合性の要因は花粉中のフラボノール配糖体および柱頭に含まれる配糖体分解酵素の差異にあると結論して，学界の注目を引いた．これと相前後して，*Forsythia* 属各種の花についてもフラボノイドの調査が行なわれている．すなわち，*F. fortunei*[3]，*F. europaea*[4]，*F. intermedia*[4]，*F. ovata*[4]，*F. suspensa*[3,4] の花では Rutin のみが，また *F. viridissima*[5] の花では Rutin のほかに Quercitol-rhamnoglucoside の存在が報告された．したがって同属のチョウセンレンギョウの花にも Rutin の含まれることは想像に難くないが，これについてはまだ文献に記載がないから，われわれの実験結果を一応報告することにした．

なお，上に述べた Kuhn, Moewus らの所論については，その後 K. Esser u. J. Straub (1954)[6] が実験に再現性のないことを指摘し，次いで H. Reznik (1957)[7] は Moewus らの実験植物と全く同一の株* を用いて慎重に追試した結果，問題の 2 種の不和合植物の花粉ではフラボノイド成分には差がなく，いずれも Rutin, Kaempferol 3-glucoside および Chlorogenic acid の含まれることを示して，当初の結論に反論した．さらにその後の O. Renner (1958)[8] の論文によってもフラボノイドと不和合性との間には関連性のないことが強調された．

実験の部

Rutin (Quercetin-3-rhamnoglucoside): 緑色部分を除いた新鮮花 52 g を 90% エタノール 200 ml で室温下に 1 夜浸漬後，圧搾沪過し，残滓をさらに 2 回同様に冷浸して得た抽出沪液を合わせ，減圧濃縮して 25 ml とし，ほぼ等量ずつの石油エーテルおよびベンゼンで順次振盪したのち水層を放置すると，淡黄色針状結晶が球塊となって析出する．収量，0.25 g，収率 0.48%．これを希エタノールから数回再結する．Mp. 191〜200°，$FeCl_3$ で暗緑色，Mg-HCl で紅色．n-BuOH/AcOH/H_2O (4 : 1 : 5) および 25% AcOH によるペーパークロマトグラムでは，それぞれ Rf 0.40; 0.67 (Rutin ではそれぞれ Rf 0.41; 0.69)．沪紙電気泳動〔M/10 硼砂 (pH 9.4)，400〜500 V，12 mA/cm，東洋沪紙 No. 50〕では 2 時間後に陽極側へ 46 mm (Rutin では 45 mm) だけ泳動する．元素分析**: 実験値 C 48.72,

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan. 東京教育大学理学部植物学教室

** Research Institute for Natural Resources, Shinjuku, Tokyo, Japan. 資源科学研究所

* Heidelberg 大学の植物園に栽植されているもので，Esser u. Straub もこの株について実験した．

** 元素分析はすべて薬理研究所の大畑大次郎氏による．

H 5.23%. $C_{27}H_{30}O_{16}\cdot 3H_2O$ としての計算値 C 48.79, H 5.46%.

加水分解: 上記の無水物 148.1 mg を 3% H_2SO_4 10 ml 中で直火で 30 分間煮沸し，冷後アグリコンの結晶を沪集する. 無水物として 75.5 mg. $C_{27}H_{30}O_{6} \rightarrow C_{15}H_{10}O_{7}$ としての計算値 49.51%; 実験値 50.98%. 希エタノールより再結，黄色針状結晶，Mp > 300°, メタノール溶液は $FeCl_3$ で暗緑色, Mg-HCl では紅色を呈する. *n*-BuOH/AcOH/H_2O (4 : 1 : 5) によるペーパークロマトグラムでは Rf 0.76 (Quercetin は Rf 0.77), 元素分析: 実験値 C 53.30, H 4.23%. $C_{15}H_{10}O_{7}\cdot 2H_2O$ としての計算値 C 53.26, H 4.17%.

Quercetin pentaacetate: 上記のアグリコン 50 mg を無水酢酸 2 ml, ピリジン 3 滴とともに沸騰水浴上に 1 時間加熱後水中に投じ，固化物を含水エタノールから再結. 無色長針状結晶 (純品 34.6 mg), Mp. 193～4°. Quercetin pentaacetate と混融しても融点は降下しない.

カリ熔融: アグリコンの小量を KOH とともに 220～230° で 10 分間熔融し，熔塊を水にとかして塩酸酸性としてエーテルで抽出，エーテル層を重曹水で振って，反応生成物を有機酸区分とフェノール区分とに分別する. 後者は松材反応陽性で，フロログルシンの存在を示す. 各区分についてペーパークロマトグラフを行なうと，フロログルシンおよびプロトカテク酸が明瞭に検出される. (上表参照)

		BuOH/AcOH/H_2O (4 : 1 : 5)	BuOH/Pyridine/NaCl aq. (飽和) (1 : 1 : 2)	Anisidine による呈色
カリ熔融生成物	フェノール区分	0.80	0.94	淡褐
	酸区分	0.90	0.86	赤褐
対照	フロログルシン	0.81	0.95	淡褐
	プロトカテク酸	0.90	0.86	赤褐

結合糖の証明: 上記の加水分解母液を中和して真空濃縮し，その一部をとり常法によって Phenylosazone をつくる. 析出物を鏡検すると黄色針状結晶と黄色針晶束とが混じているから，冷アセトンで可溶部と不溶部とに分け，それぞれ希エタノールから再結する. 前者は Mp. 190～191° で L-Rhamnoosazone, 後者は Mp. 207～9° で D-Glucosazone と同定された.

なお，糖液の残部は真空で乾涸し，温メタノールで抽出し，それについてペーパークロマトグラフを行なって D-Glucose と L-Rhamnose とを再確認した.

	Rf*	Resorcinol による呈色	Aniline hydrogen phthalate による呈色
配糖体の糖成分	0.20	褐	褐
	0.40	褐	褐黄
対照 D-Glucose	0.21	褐	褐
対照 L-Rhamnose	0.40	褐	褐黄

* *n*-BuOH/AcOH/H_2O (4 : 1 : 5) で展開

文 献

1) Kuhn, R., und Löw, I., Chem. Ber. **83**: 474 (1949). 2) Moewus, F., Biol. Zbl. **69**: 181 (1950). 3) Nagahski, J., Porter, W.L., and Couch, J.F., Journ. Amer. Chem. Soc. **69**: 572 (1947). 4) Sosa, A., and Plouvier, V., Bull. Soc. Chim. Biol. **30**: 273 (1948). 5) ——, and ——, ibid. **30**: 266 (1948); Compt. rend. **226**: 955 (1948). 6) Esser, K., und Straub, J., Biol. Zbl. **73**: 449 (1954). 7) Reznik, H., ibid. **76**: 351 (1957). 8) Renner, O., Z. Naturforsch. **13 b**: 339 (1958).

Short Communication

Michio Ito*: Complete Regeneration from Single Isolated Cells of Fern Gametophyte.

伊藤道夫*: 単離されたシダ配偶体細胞の再生

Received May 19, 1960

Although it has been for long reported that single cells isolated from plant tissue were able to grow to somewhat organized tissue, there are as yet, so far as the writer knows, only two references[1,2] to the complete regeneration to whole plant from single isolated cells, except for some coenocytic lower plants. However, such a case reported by Meyer[1] in the fern gametophyte was merely a spontaneous accidental one. Whereas, the present writer has succeeded to bring about voluntarily the mature gametophyte from the optional single isolated cell of fern gametophytes in any developmental stages by an operative method (a part of results was read at the Annual Meetings in 1956 and 1958 of the Botanical Society of Japan).

As materials, gametophytes of *Pteris vittata*, *Dryopteris erythrosora* and others were used. Here the principal results, exclusively in *Pteris vittata*, will be reported. Single cells were isolated from the protonema portion and monocell-layered portion of prothallium, where the cells are over 30μ in length, but not from the meristematic region in which the cell length is very short (under 30μ). Isolation was carried out by killing the surroundings of a given cell, namely by pricking them with a fine glass needle by free hand under a binocular microscope. Culture conditions: on agar-Knop's medium, under a white fluorescent lamp, at 26–27°.

Every single isolated cell regenerates and grows to a mature gametophyte, assuming the same pattern as in the normal development from spores; but the protonema portion of regenerated gametophytes is shorter and consists of smaller cells than the normal. Time lapse required for beginning of regeneration is closely related to how old the concerned portion is and how advanced in development the concerned gametophyte is: 1) In gametophytes of a definite stage of development, single cells isolated from the older portion regenerate earlier than those from the younger. In mature stage the oldest portion is the protonema, the youngest is the meristematic region; while the older portion had ceased to proliferate, those of the younger do continue it more intensely. Thus, it was demonstrated that there is an apparent gradient from the portion near meristematic region to the protonema, i.e. from apical to basal, as to the time for beginning of regeneration. 2) On the other hand, cells isolated from a given portion of gametophytes in younger stage regenerate earlier than those from the *same* portion of *other* gametophytes in older stage.

Further, it was found that even a single isolated cell is able to bear the antheridium, when it is cultured in "old" medium which contains the so-called antheridium promoting factor (or substance) detected by Döpp[3]).

References

1) Meyer, D. E., Planta **41**: 642 (1953). 2) Steward, F. C., Mapes, M. O., and Smith, J., Amer. J. Bot. **45**: 705 (1958). 3) Döpp, W., Ber d. Deut. Bot. Ges. **63**: 139 (1950).

* Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan. 名古屋大学理学部生物学教室

抄　　　録

形 態 形 成 物 質

(A) Werz, G., Weitere Untersuchungen zum Problem der Kernaktivität bei Gesenktem Zellstoffwechsel. Planta, **52**: 528–533 (1959).

(B) ――――, Über Polare Plasmaunterschiede bei *Acetabularia*. Planta, **53**: 502–521 (1959).

さきに Hämmerling はカサノリ *Acetabularia* において，その単細胞の茎の先端に特異的なカサが形成するにさきだって，その形成予定部域には核から由来するある物質が集積することをたしかめ，これに形態形成物質 (morphogenetische Substanz) となづけた．今回著者は (A) 論文において生長しつつあるカサノリの茎を基部から 10 mm のところから先端を切りすて，核をふくむ残部の切口における再生をしらべた結果主につぎのことが明らかになった．(1) 明条件におけるよりも14日間の暗処理をしてから光をあてた方がよりすみやかにカサを形成する．したがって形態形成物質は光合成の産物ではない．(2) 暗処理のかわりに trypaflavin (10^{-3}g/m*l*) 処理をおこなうと，暗処理よりもよりすみやかにカサを形成する．したがって形態形成物質は trypaflavin の存在で促進される．(B) 論文では生成しつつあるカサノリを Carnoy で固定後 pH 2 において酸性色素 Azocarmine B で染色した結果を報告している．(a) 生長のさかんな部位ではこの色素に特異的に親和性をもつ粒子が密集している．(b) 生長していないところには粒子がすくない．Microphotometry によると波長 546 mμ の吸収率が生長点で最も高く，基部へむけて勾配をなす．(c) 定性分析によってこの粒子はある種のたん白であることがしられた．(d) 粒子はおそらく形態形成物質か，あるいはその生産物と考えられる．(中沢信午)

アオミドロ葉緑体へのチミジンの結合

Stocking, C. R., and Gifford, E. M., Jr., Incorporation of Thymidine into Chloroplasts of *Spirogyra*. Bioch. Biophys. Res. Comm. **1** (3): 159—164 (1959)

緑色植物における核―細胞質相互作用という問題のうち葉緑体の合成機能における核酸の役割に関してはまだ未解決の分野である．葉緑体には微量だが意味ありげな核酸の存在がすでに幾つかの報告で証明されている．標識チミジンが DNA 生合成の効果的前駆物質であることはよく知られているが，アオミドロの培養液に 10 μc の H^3–thymidine を 3×10^{-5} M に添加し，フォルムアルデヒド・プロピオン酸・アルコールで固定，オートラジオグラフ及びヘマトキシリン発色標本を比較し，葉緑体に DNA 合成が起り得るかどうかを検した．その結果は放射活性は核よりもむしろ葉緑体に強く結合していることが示された．フィルムに接触している面積は総計 0.016 mm² でそのうち葉緑体はほぼ 0.005 mm² で 1/3 にすぎないが全放射活性の 81～94 % が葉緑体に含まれる．核は95時間後ですら特には標識されない．この活性の大凡半分は無標識媒液に入れかえても置換されず恐らく核酸に合成されたと思われる．Brachet (1959) はカサノリでチミジンが細胞質（明らかに葉緑体）に結合したことを報告し，Plaut と Sagan (1958) もアミーバで核はフォイルゲン反応が弱く，チミジンは細胞質に結合すると報告している．このアオミドロはフォイルゲン反応負であることが知られているが，（訳者註：この点についてはすでに新家等 (1956) が核酸がないのではなく蛋白干渉現象のせいであることを明らかにしている）これらの事実はアオミドロでは葉緑体に特殊な核酸代謝があることを示している．しかし一方これら原始的生物では果してチミジンが絶対的先駆物質であるのかどうかをなお結論の前に再検討してみる必要があるかもしれない．(吉田吉男)

Bot. Mag. Tokyo **73**: 269-277 (July 25, 1960)

Studies on the Dehydration Resistance of Higher Plants III Discussions on General Analysis Focussed on the Dehydration Resistance of Pine Yearlings

by Tadayoshi TAZAKI*

Received December 11, 1959

In previous papers[1,2] the dehydration resistance of higher plants was analysed by some mathematical formulae. The analysis was divided into two parts, general and special. In the present paper some discussions on general analysis were undertaken at first on the dehydration resistance of pine yearlings and then on their place among other higher plants. Before entering the main subject the results of the previous paper[2] will be summarised. Thus, in general analysis three solutions of the equation of water economy were led for determining dehydration resistance ($\boldsymbol{t}$). In all cases graphical solution of equation (12)** for t gives the value of $\boldsymbol{t}$, and when inequality (13)** is fulfilled,

$$\boldsymbol{t}=\frac{1}{C}\left(\frac{6000D}{d}-\frac{A}{k}\right), \qquad (1)^{***}$$

where A and C are, respectively, the initial stomatal transpiration and the final transpiration in mg./g. dry weight/hr./10 mm. Hg, k the tendency of transpiration decrease, D the lethal deficit in percentage of oven dry weight, and d the atmospheric saturation deficit in mm. Hg. Lastly in highly resistant cases the second term of equation (1), A/k, can be omitted and $\boldsymbol{t}$ is expressed as equation (15)**.

1. Discussions on the dehydration resistance of pine yearlings

An empirical formula,

$$T=Ae^{-kt}+C, \qquad (2)$$

can be applied to the time-transpiration curves of pine yearlings (Fig. 1–*A*). The values of A greatly differed by the hour of detaching shoots, the largest in the morning, decreased with time and again increased somewhat in the afternoon (see also Fig. 19 in another paper[3]), while the values of C were rather constant. The values of k in these samples, 0.0576–0.1204, must be examined in relation to other measures, because the applicability of equation (1) has much bearing on them. From equation (2),

$$k=\frac{1}{0.4343t}\log_{10}\frac{A}{T-C}\,. \qquad (3)$$

The amount, $(T-C)/A$, is the stomatal transpiration relative to the initial value. From equation (3) it is clear that the larger the relative stomatal transpiration at definite time (t), the smaller the value of k, and that the latter is not directly dependent upon the absolute values of A, C and T but upon the relative ones. For the direct comparison of time-transpiration curves it will be useful to utilize the linear relationship between t and the logarithm of the relative stomatal transpiration, illustrating the relationship in semi-logarithmic scale, from which k can easily be obtained (Fig. 1–B).

* Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan.

** Nos. of equations and an inequality in the previous paper[2].

*** Equation (13) in the previous paper[2].

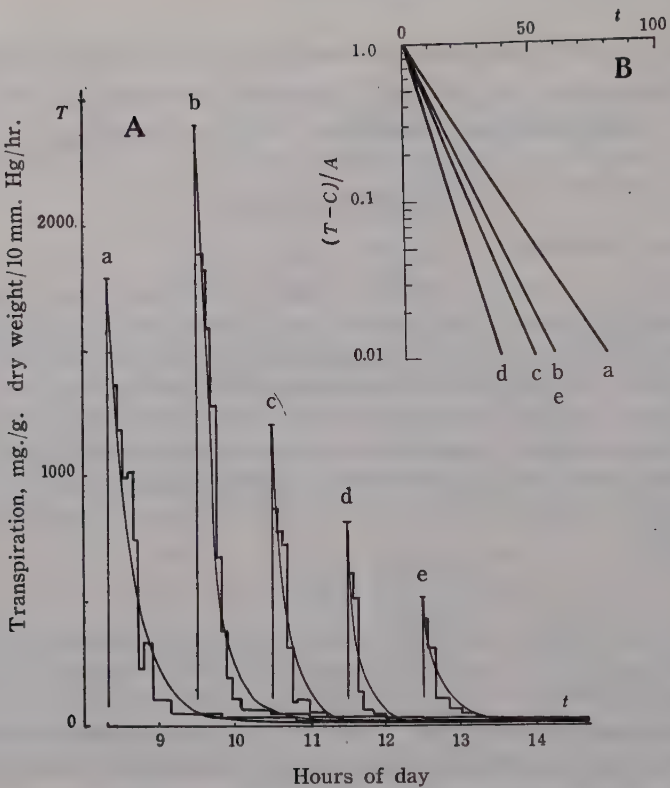


Fig. 1. A: Application of the formula, $T=Ae^{-kt}+C$, to the variation of transpiration amount after detaching shoot of pine yearlings measured on July 6, 1950. The values of A, C and k were shown in Table 1.

B. The relation between t and $(T-C)/A$ by different values of k for the curves in A.

For the calculation of dehydration resistance it will be fortunate if we can use the simple formula of equation (1) instead of troublesome graphical solution, with fulfilment of the inequality. In Fig. 2 is shown the A-k relationship for considerable numbers of summer and winter experiments. The values of k were between 0.05 and 0.12 with a few exceptions, centring in most cases around 0.08. No significant tendency was observed in the relation between k and A, except that abnormally small k appeared when A was small. The applicability of equation (1) was examined by putting the values of C, d and D into the inequality, being 20, 10 and 240 respectively. Equation (1) can be applicable to the area on the right of the curve in Fig. 2, which could cover all points including those with small k at left-lower corner of the figure.

Now, we are in a position to calculate dehydration resistance ($\boldsymbol{t}$) by equation (1). Table 1 shows the value of $\boldsymbol{t}$ calculated from the time-transpiration relationships in Fig. 1. They were between 3.9 and 6.4 days when d and D were as before — $D=350-110=240$ (Fig. 23 in another paper[3]).—In the last line of the table were shown the values of $\boldsymbol{t}$ computed by the simpler equation (15)** of the pine yearlings. They were larger than those calculated from equation (1), the difference being between 0.2–1.5 days. So, it may be concluded that equation (1) is preferable in the most cases.

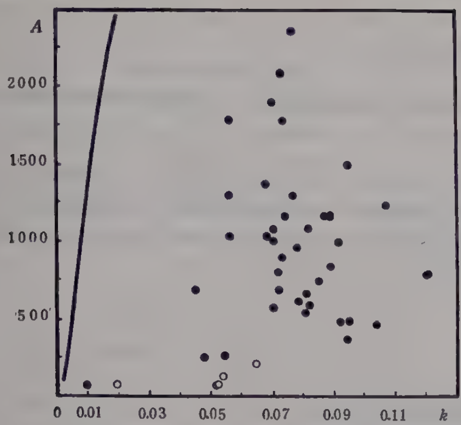


Fig. 2. The relation between A and k with possible application of equation (1) to the dehydration resistance of pine yearlings. In the area on the right of the curve, equation (1) is applicable.

●……summer experiment,
○……winter experiment.

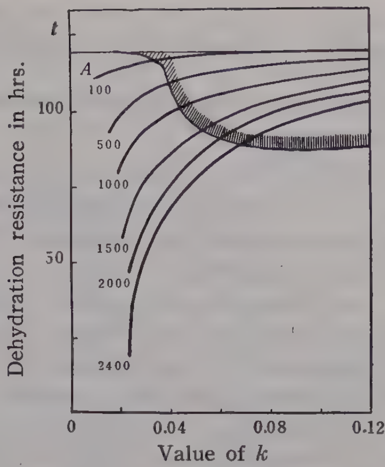


Fig. 3. Dehydration resistance (t) of pine yearlings as influenced by A and k when C, D and d are 20, 240 and 10 respectively.

From Table 1 it may be observed that the value of t varied in accordance with the values of A, C and k. On the basis of equation (1) the influences of A and k were examined by varying these two measures with constant C (=20), D (=240) and d (=10). In Fig. 3 is illustrated the k-t relationships when A was between 2400 and 100. From equation (1) it is clear that the t values in each curve approach to 6000/Cd or 120 hrs. with infinite increase of k. At every value of A, dehydration resistance (t) decreases as k becomes smaller, the remarkable decrease of t at larger values of A being worthy of notice. It must be remembered, however, that the curves outside the enclosed area in Fig. 3 never realized from A-k relationship as shown in Fig. 2. Taking this limitation into account the dehydration resistance under given condition will be between 120 and 92 hrs. and the variation range was comparatively small, in other words, the influence of A and k on the dehydration resistance was much smaller as was expected. The influences of C, D and d, however, are much striking, as these measures directly decide the value of the first term in the right side of equation (1). Therefore, the dehydration resistance of the pine yearlings is less controlled by A and k, and much by C, D and d. Moreover, under a given humidity condition (d) the fluctuation of D was much smaller than that of C, whose range was between 4 and 30 mg./g. dry weight/10 mm. Hg/hr. So,

Table 1. Calculated dehydration resistance of pine yearlings (t) by the time-transpiration curves of Fig. 1, under a saturation deficit of 10 mm. Hg.

Time at detaching shoot (hr.)	a 8.30	b 9.30	c 10.30	d 11.30	e 12.30
A	1780	2385	1185	785	480
C	20	15	15	15	20
k	.0576	.0767	.0866	.1204	.0767
t by equation (1), hrs.	94.3	125.0	145.0	153.0	115.0
t by eq. (15)**, hrs.	120.0	160.0	160.0	160.0	120.0

it must be no exaggeration to say that the dehydration resistance of pine yearlings is mainly controlled by the cuticular transpiration, C.

2. The place in dehydration resistance of the pine yearlings among other higher plants

Are the results obtained in the pine yearling also applicable to other higher plants? To answer this question we must take up another plant of different life type and compare its dehydration resistance with that of the pine yearlings using the same procedure.

For this purpose the author intends to examine again the dehydration resistance of "summer cut" mulberry plants, for which special analysis has been applied in a previous paper[2]). The dehydration resistance of this plant was 2.6 hrs. in normal leaves and 0.6 hr. in "dull" ones, which was far smaller than that of the pine yearlings. This difference was examined by drawing the time-transpiration curves of mulberry plants on a dry weight basis in order that they can be directly compared with that of the pine yearling (Fig. 4). The amount of initial transpiration ($A+C$) was somewhat larger in the former, but not so different from that of the latter, and the tendency of transpiration decrease (k), though considerably small in older mulberry leaves, was yet in the range of fluctuation in the pine yearlings. Different was the final transpiration (C); the mulberry leaves, even normal ones, transpired ten times heavier than the pine yearlings. This value can be considered as the cuticular transpiration in usual leaves, because their stomata close completely after detaching. In "dull" leaves with imperfect closure of stomata, however, C was indeed forty times as large as that of the pine yearlings. So the difference in dehydration resistance between both plants does not derive from A and k, but from C. Besides, the small value of lethal deficit (D) in the mulberry leaves may play some part.

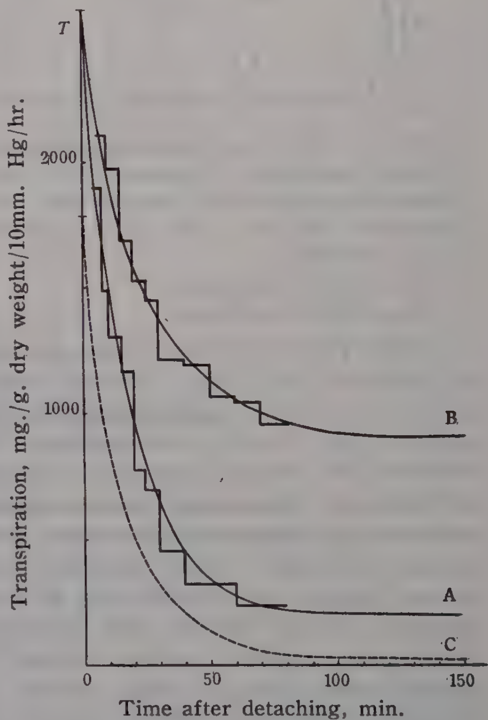


Fig. 4. Comparison of pine yearling with mulberry leaves in the time trend of transpiration amount after detaching shoots or leaves. A. The 4th leaf (normal) of a "summer cut" shoot, detached at 12.04 hr. on Aug. 3, 1952. $T=2390e^{-0.0575t}+200$. B. The 10th leaf of the same shoot, detached at 9.13 hr. on Aug. 6, 1952. $T=1690e^{-0.0427t}+900$. C. Pine yearling, a) in Fig. 1. $T=1780e^{-0,0576t}+20$.

For other plants no complete data can be available of all measures for the calculation of dehydration resistance. Fukuda[4]) applied a similar empirical formula to the author's to the experimental results of Pfleiderer[5]) concerning the depression in transpiration of detached leaves, but their dehydration resistance can not be calculated for lack of data in saturation deficit and lethal deficit. Only k and A/C can be compared provided that the humidity condition had been maintained nearly

constant during the experiment. In Fukuda's formula, $T_t+K=A''e^{-kt}+C''$, the left term T_t+K corresponds to T of our equation (2), and signs A'' and C'' were used by the present author as humidity relation was obscure in Fukuda's paper. Besides, $3t/10$ must be put in the place of t in Fukuda's equation to convert the time unit to 1 min. The calculated values of k, A'' and C'' were tabulated in Table 2. The

Table 2. The values of k converted from Fukuda's[6]) equation, $T_t+K=A''e^{-kt}+C''$. t must be replaced by 3t/10 if minutes should be used as time unit. The signs, A'' and C'' were used instead of A and C as humidity relation was obscure in his paper.

Species	Condition	k	A''	C''
Sambucus nigra	Outdoors, fine day. Aug. 11.	0.1800	60.0	9.0
	Outdoors, fine day. Aug. 10.	0.1320	14.1	3.1
	Outdoors, fine day. Aug. 10.	0.1155	12.2	3.4
Lysimachia ciliata	June 13 in dark room supplied by 500W lamp 1m. apart	0.0237	1.1	1.6
		0.0768	1.1	2.6
		0.0816	2.0	3.2
Atriplex hortensis	The same condition as above	0.0624	4.0	4.5
		〃	3.2	2.6
		〃	0.7	2.1
Chenopodium album	The same condition as above	0.1140	3.0	2 8
		〃	2.4	2.0
Stachys germanica	The same condition as above	0.0990	3.0	2.8
		〃	1.9	2.2
Picea excelsa	The same condition as above	0.0339	2.6	0.55
		〃	4.6	1.0
		〃	1.3	0.8

values of k fall within the same range of those in both plants discussed in the foregoing and the small values of A'' in dark-room experiments may be due to the insufficient opening of stomata under such a gloomy condition. Fukuda also applied to some of the transpiration courses in detached leaves another empirical formula, $T_t+K=A''e^{-kt^2}+C''$, the curves by this formula being inverse S type. This formula, according to Fukuda, fitted well to young or soft leaves intensively transpiring outdoors. Concerning pine yearlings the formula was better fitted in some cases, especially when transpiration decrease was slow immediately after detaching. In these cases more precise values of dehydration resistance will be obtained if we analyse it by this formula, as the application to this case of equation (2) gives somewhat higher value to A and consequently the calculated transpiration in the earlier stage becomes too high. Definite integral for arbitrary range of e^{-kt^2}, however, is only possible by numerical integration, and the analyses by this function will be left to future studies.

Monsi[6]) measured the variation course of transpiration in detached leaves of *Fatsia japonica*, an evergreen shrub, in December. An empirical formula applied by the author to one of his data was $T=412.5\,e^{-0.0794\,t}+37.4$, which coincides fairly well in A and C with the author's unpublished data in *Quercus myrsinaefolia*, an evergreen oak, during winter period, i.e., $T=395\,e^{-0.0512\,t}+25$. In passing, a summer result of the same plant was $T=515\,e^{-0.0808\,t}+15$, different from our pine yearlings only in small value of A.

Directly comparable with our pine yearlings were the measurements of transpiration (mg./g. dry weight/20 mm. Hg/min.) by Satoo[7]) at early autumn in detached shoots of the 5-month-old yearlings of *Cryptomeria japonica*, *Chamaecyparis obtusa* and *Pinus densiflora*, pot-cultured with Kantô loam. Empirical formulae applied to his results by the present author are, $T=393\,e^{-0.0421\,t}+72$ for *Cryptomeria*, $T=345\,e^{-0.0339\,t}+30$ for *Chamaecyparis* and $T=417\,e^{-0.0434\,t}+18$ for *P. densiflora*. The values of C in *P. densiflora* conform well to those of our pine yearlings, *P. Thunbergii*, and A, k are somewhat smaller. Also he determined the lethal water content in average of 168, 85 and 105% on an oven dry basis in *Cryptomeria*, *Chamaecyparis* and *P. densiflora* respectively. The value in the third species conform well to the lethal water content of *P. Thunbergii* at the middle of July (see Fig. 21 in another paper[3])), but was higher than that at the end of August (88%). The water content immediately after detaching were 572, 450 and 464%, so lethal deficit will be 404, 365 and 359% respectively. As the inequality (13)** is fulfilled in each case, we can calculate their dehydration resistance by equation (1). Thus, calculated values of dehydration resistance were 54, 109 and 222 hrs. respectively. The value of *P. densiflora* was over twice as much as those of our *P. Thunbergii*, but it must be remembered that the water content of those conifers measured by Satoo in September belonged to comparatively large one, perhaps by culture conditions, for the values by the same author in July and August of that year were much smaller even in well watered condition. If we calculate the dehydration resistance by the water content of 350, 250 and 220% at the end of August, it was 23, 49 and 55 hrs. for *Cryptomeria*, *Chamaecyparis* and *P. densiflora*. Here the value in *P. densiflora* was only a half in *P. Thunbergii*. In conclusion, it is quite certain that the order in dehydration resistance of these three genera is as follows:

Cryptomeria < *Chamaecyparis* < *Pinus*.

From the comprehensive data by Monsi[6]) in various plant species of Japan the present author has calculated the values of A and C tabulated in Table 3. The values of C in evergreen broad-leaves fall in most cases between 20 and 60 mg., somewhat larger than our pine yearlings, while in deciduous broad-leaves they were much larger, i.e., 120-360 mg., our mulberry plant belonging to this group. The values in Gramineae species seem somewhat smaller than deciduous broad-leaves and the largest values are found in water plants followed by juicy herbs such as *Vicia*, *Commelina* and *Mirabilis*, while a leaf-succulent species, *Sedum*, showed one of the minimum values. So the order of dehydration resistance, if decided only by the value of C, will be,

Pine yearlings > Succulent sp. > Evergreen broad-leaves > Gramineae sp. >
Juicy herbs > Water plants.

As for A, evergreen broad-leaves have comparatively small values below 1000, and deciduous broad-leaves and Gramineae species show medium values of 1000–2000, out of which barley and wheat are the exceptions, the values being the same as or a little smaller than water plants and juicy herbs. The pine yearlings have comparatively large values of A at least in summer.

It will be of interest to examine the dehydration resistance of various plants from interrelationships between A, C, k and D. Under conditions of 10 mm. Hg saturation deficit and t in hour, equation (1) will be,

$$t=\frac{10D}{C}-\frac{A}{60CK}\,. \tag{4}$$

Table 3. Calculated values of A and C in various plants from Monsi's paper.[5])

Species	Date	A	C	Water content, oven dry basis
1. Evergreen trees and shrubs:				
Fatsia japonica	May 19	1034mg.	119mg.	312%
	Aug. 15	530	45	200
	Oct. 16	488	21	193
	Feb. 13	220	25	173
Pittosporum Tobira	Aug. 28	528	21	178
	Jan. 21	295	8	144
Laurus nobilis	Sep. 13	835	28	117
Rhododendron hortense	June 25	1016	46	156
Daphne odora	Aug. 7	1019	69	270
	Feb. 1	484	46	223
Evonymus japonicus	May 6	1383	76	234
	Aug. 23	982	41	213
	Jan. 15	487	17	178
Thea sinensis	Mar. 24	698	24	157
Camellia japonica	Apr.	314	31	113
Ilex integra	Apr.	572	24	138
Hedera japonica	Aug. 27	529	73	157
Torreya nucifera	Aug. 27	479	35	163
2. Deciduous trees and shrubs:				
Aphananthe aspera	Sep. 12	1303	119	164
Kerria japonica	June 20	2292	248	212
Sambucus Sieboldiana	June 15	2268	138	335
	Aug. 17	1451	140	300
	Oct. 10	1332	358	292
Cornus controversa	June 21	1012	196	203
3. Herbs:				
Dioscorea japonica	Sep. 24	1346	82	400
Pleioblastus Simoni	Sep. 6	1536	160	122
Phragmites communis	Sep. 11	1472	126	163
Trachycarpus excelsus	Aug. 20	547	67	120
Triticum sativum	Mar. 10	3230	210	400
Hordeum sativum	Mar. 26	4472	99	525
Mirabilis Jalapa	July 19	2718	450	733
Vicia Faba	Jan. 28	5459	1069	808
Erigeron canadensis	Aug. 16	2646	216	400
Commelina communis	June 14	5219	851	808
Jussiaea repens	July 23	3987	2179	426
Hydrocharis asiatica	July 23	366	2413	669
Sedum alboroseum	Aug.	756	20	1150

Omitting the second term of the right side, t–D relationship becomes linear at definite values of C (Fig. 5). In this condition the dehydration resistance of our pine yearlings falls within the area surrounded by solid lines, for which C and D are 10–20 and 170-240 respectively. Taking into account the second term, t will be smaller by $A/60CK$ than the former case. Deducting the maximum value of the second term, $2400/(60\times0.05\,C)$, the area is enlarged to that surrounded by broken lines and the range of dehydration resistance will be 45–240 hrs. The range of dehydration resistance of younger leaves in "summer cut" mulberry was only 2–8 hrs. for the water content of 250–300%, far smaller than that of pine yearlings. For other plants the ranges or points were plotted according to Monsi[6]) and Satoo[7]). As the values of lethal deficit were not shown in Monsi's paper the author assumed the lethal water content to be a half of the usual water content in the light of Pisek and Berger's[8]) data. From Fig. 5 the dehydration resistance of various plants is clearly shown in relation to D and C. The mightiest of all is *Sedum* with maximum D and C. Coniferous yearlings are resistant with small value of C but their D is

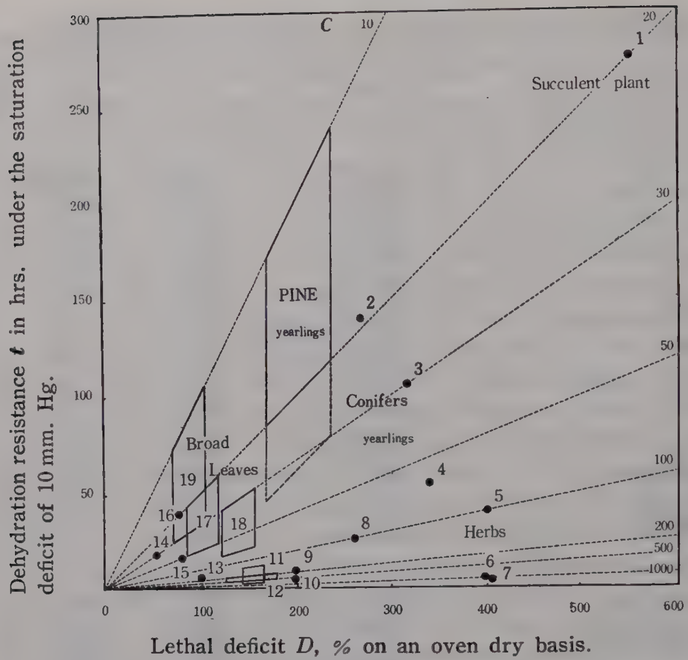


Fig. 5. Dehydration resistance (*t*) of various plants in relation to lethal deficit (*D*) and cuticular transpiration (*C*). 1. *Sedum*, 2. *Pinus densiflora* yearling, 3. *Chamaecyparis* yearling, 4. *Cryptomeria* yearling, 5. *Impatiens*, 6. *Commelina*, 7. *Vicia*, 8. *Hordeum*, 9. *Triticum*, 10. *Erigeron*, 11. *Sambucus*, 12. *Morus*, 13. *Cornus*, 14. *Camellia*, 15. *Phragmites*, 16. *Thea*, 17. *Fatsia*, 18. *Daphne*, 19. *Pittosporum*.

much smaller than the succulent species. Next resistant is evergreen broad-leaves with small *C* and *D*. Most susceptible are deciduous broad-leaves and herbs due either to small *D* or to large *C*.

3. Conclusion

So far the value of dehydration resistance was calculated under the saturation deficit of 10 mm Hg, but it can be calculated under arbitrary saturation deficit if we only put concerned values instead of 10 mm. Hg. This kind of dehydration resistance above mentioned rarely occurs in intact leaves during drought condition, for water supply from soil gradually, not abruptly, decreases as soil water content approaches to wilting percentage, and even if the water supply is practically cut by the drying of the soil below wilting percentage, the leaves can absorb water for a while from axial part of the plant, as a great deal of water is stored in stems and petioles. So, water absorption as well as transpiration must be taken into account in such cases, the investigation of drought resistance becoming more complex. However, in small plants such as our pine yearling the cessation of water entrance into leaves follows soon after the cessation of water supply from soil, as the mass of stem and root are too small to maintain the water supply to leaves. For this case our investigation method of dehydration is directly applicable to its drought resistance. But it must be remembered that when soil water content in the rhizosphere decreases to wilting percentage, the amount of transpiration diminishes to the level of cuticular

transpiration, the shoot water content decreasing to some extent. In this case equation (15)** can be applicable without reservation, and by this way the drought resistance in the pot-cultured yearlings of three conifers was investigated by Satoo[7]), and that of the black-pine at sand dune regions by the present author[3]).

Summary

Some discussions were put forward on general analysis of the dehydration resistance in pine yearlings and then on their place among higher plants as to this character.

1. It was substantiated that the simplified solution of the equation of dehydration resistance can be applicable to all cases in pine yearlings.

2. The influence of initial transpiration and of tendency of decreasing transpiration upon the dehydration resistance were examined when cuticular transpiration and lethal deficit were given. These influences were not so serious as was expected at least in the case of pine yearlings.

3. The difference in dehydration resistance between pine yearlings and mulberry plants proved to be due to the difference in cuticular transpiration.

4. The pine yearlings and mulberry plants were compared with other higher plants in cuticular transpiration, initial transpiration, tendency of decreasing transpiration, lethal deficit and dehydration resistance. The pine yearlings belong to one of the most resistant species, while the mulberry plants one of the most susceptible ones.

The author wishes to express his cordial thanks to Prof. M. Monsi, the University of Tokyo, and Prof. K. Hôgetsu, Tokyo Metropolitan University, for their kind advice and criticism throughout this investigation. Thanks are also due to Messrs T. Ushijima and T. Murakami to their help for preparing the text.

References

1) Tazaki, T., Bot Mag. Tokyo **73**: 148 (1960). 2) ——, ibid. **73**: 205 (1960). 3) ——, Jap. Journ. Bot. **17** (2): 239 (1960). 4) Fukuda, Y., Pflanzenforschung Heft **19**: 1 (1933). 5) Pfleiderer, H., Zeitschr. f. Bot. **64**: 303 (1933). 6) Monsi, M., Jap. Journ. Bot. **14**: 97 (1944). 7) Satoo, T., Bull. Tokyo Univ. Forests No. 51: 1 (1956). 8) Pisek, A. und Berger, E., Planta **23**: 124 (1938).

摘　要

田 崎 忠 良: 高等植物の乾燥抵抗に関する研究 III

クロマツ苗を中心とする乾燥抵抗の考察

この報告ではクロマツ苗の乾燥抵抗を考察し，ほかの植物の乾燥抵抗との関係を論議した.

1. 乾燥抵抗に関する簡便式はクロマツ苗において，すべての場合に適用できることがわかった.

2. 一定のクチクラ蒸散・致死飽差の下で， 乾燥抵抗に対する最初の蒸散量・蒸散減少度の影響を調べた結果，その影響は少なくともクロマツ苗の場合には想像されるより著しく少ないことがわかった.

3. クロマツ苗と栽培グワの乾燥抵抗の差は，クチクラ蒸散に原因する.

4. クロマツ苗と栽培グワの乾燥抵抗・クチクラ蒸散量・最初の蒸散量・蒸散減少度および致死飽差を，ほかの高等植物と比較した. その結果クロマツ苗は乾燥抵抗が最も強い植物の一つであり，栽培クワは最も弱い植物の一つであることがわかった.（東京農工大学繊維学部）

Bot. Mag. Tokyo, **73**: 278—282 (July 25, 1960)

Developmental Studies in the Genus *Polygonum*. I. Microsporogenesis of *Polygonum persicaria* L.*

by Yukio DOIDA**

Received December 21, 1959

The author has studied the developmental process of microspores from the stage of formation of anther primordia in the genus *Polygonum* which, taken in wider sense, includes *Fagopyrum*. The author's previous studies showed that the number of pollen grains formed in a single pollen sac is very small and is a constant specific trait. Accordingly, the *Polygonum* species could be classified into 5 types based on the number of pollen grains per sac[1, 2, 3]). Those are types with (1) 8, (2) 16, (3) 32, (4) 128 and (5) 256 pollen grains in one pollen sac.

Nakai[4, 5]) studied the Polygonaceae growing in Japan, and classified them into six genera: *Polygonum*, *Fagopyrum*, *Rheum*, *Rumex*, *Koenigia* and *Oxyria*. Furthermore, he studied in detail the genus *Polygonum* and divided it into several sub-genera, of which one was further divided into sub-sections. Hedberg[6]) studied the genus *Polygonum* and classified it into 9 types based on pollen morphology. Nakai[5]) and Ikuse[7]), however, reported that the genus *Polygonum* should be classified into 2 major groups on this basis.

P. persicaria used in this study is placed by Nakai in section *Persicaria eupersicaria* Gross of sub-genus *Persicaria* Tournefort.

In the present paper the cytological and histological aspects of microsporogenesis of *P. persicaria* which does not fall into any of the above five types are reported. Moreover, the author's investigation disclosed an interesting relation between his five types of pollen grain number per sac and pollen grain morphology.

Materials used in the present study were collected in Misima, Sizuoka Prefecture. The process of microsporogenesis was observed by means of the usual paraffine section method. The inflorescences were collected in various developmental stages and fixed in Carnoy's or Farmer's fluid. Heidenhain's iron-alum hematoxylin, Feulgen reaction and Lillie's reagent for polysaccharide staining were used for staining. Mature pollen grains were observed by the acetocarmine squash method.

Observations

Normal development of anthers

A cross section of a very young anther of *P. persicaria* shows a mass of homogenous meristematic cells surrounded by the epidermis (Figs. 1, 2). Following the division of the meristematic cells, the young anther becomes slightly four-lobed (Fig. 3). In each of the lobes, an archesporial cell differentiates from the subepidermal layer. This cell is the future pollen mother cell. It gradually increases in size without any mitotic division until meiosis sets in (Fig. 4). Meanwhile, cells surrounding the only pollen mother cell carry out active mitotic divisions. Then they differentiate into three layers, namely the outermost "epidermis", the adjacent

* Contribution No. 321 from National Institute of Genetics, Misima.

** Biological Institute, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya, Japan. Present address: National Institute of Genetics, Misima, Sizuoka-ken, Japan.

"endothecium" and the innermost "transitional" cell layer (Fig. 5).

Tapetal cells which have an important function in the maturation of pollen grains differentiate toward the center from the transitional layer (Fig. 5). In this stage each pollen sac consists of four cell layers enveloping the pollen mother cell (Figs. 6, 7). After the differentiation of the tapetum, the transitional layer becomes threadlike and soon disappears.

To the author's knowledge in all other higher plants in which the process of microsporogenesis is well known, the archesporial cell goes through mitotic divisions resulting in a number of pollen mother cells. But in *P. persicaria* the archesporial cell assumes the function of the pollen mother cell without any further mitosis. Therefore, only one pollen mother cell is formed in a pollen sac (Fig. 7).

Prior to the meiotic division of the pollen mother cell, the tapetal cells perform mitotic divisions without cytokinesis. Binucleated tapetal cells are the result (Fig. 8). Tetra-nucleated tapetal cell resulting from successive mitotic divisions or tetraploid or higher-ploid cells following endomitosis were not observed in this species.

The meiotic process of the pollen mother cell is very regular. Table 1 shows the result of the observation of meiosis. Abnormal division is scarcely seen. As a result of meiosis, tetrads of linear type are formed, i.e. four microspores are arranged in a row (Fig. 14), but some of the tetrads are Y– or X–shaped (Fig. 15).

After the meiotic process, the tapetal cells become hypertrophied (Fig. 17), vacuolated and then they gradually degenerate. Each mature pollen sac consists of two cell layers enclosing 4 pollen grains (Figs. 21, 29).

Table 1. Observation of Meiotic Process (*P. persicaria*)

	Meiotic Stage						
	Pro-phase	Meta-phase I	Ana- to Telo-phase	Inter-phase	Meta-phase II	Ana- to Telo-phase II	Tetrad
Normal	18	11	24	2	0	25	28
Abnormal	0	1	0	0	0	0	2

Table 2. Number of Pollen Grains in a Pollen Sac (*P. persicaria*)

	Nos. of Pollen Grain					Total	Mode
	4	8	12	3+2m*	4+1m*		
Nos. of Cases (1958)	248	20	0	1	0	269	4
Nos. of Cases (1959)	268	41	0	0	2	311	4

* m: dwarf pollen grain

Deviations from the regular development

1) Pollen sacs having eight pollen grains are also found, but their frequency is small (Table 2). The process of microsporogenesis in this case is shown in Fig. 23–28. It is not essentially different from the process of microsporogenesis observed in *P. nodosum*. The author reported in a previous paper[1]) that this type of pollen grain formation represents type 1. An archesporial cell divides mitotically once and the daughter cells assume the function of pollen mother cells (Fig. 23). Thus, two pollen mother cells are produced in a pollen sac (Fig. 24). Meiosis proceeds regularly in

each of the two pollen mother cells. Linear arrangement of tetrads is not found in this case.

2) Abnormal divisions are one of the causes of abnormal pollen formation. Some pollen sacs have dwarf pollen grains. Those are perhaps produced by lagging chromosomes or micro-nuclei. However, such aberrations are very rarely seen (Tables 1 and 2).

3) In this species, formation of empty pollen sacs was observed (Figs. 29, 30). This phenomenon is commonly considered to be due to (1) lack of differentiation of the pollen mother cell or (2) its degeneration. In many cases a pollen mother cell is differentiated in each lobe of a young anther, but the surrounding tissue does not fully develop. Especially, failure of the tapetal tissue to develop may be the cause of pollen degeneration or empty pollen sac formation. Fig. 31 shows a case of this type.

Pollen Grains

As shown in Fig. 21, the pollen grains of *P. persicaria* belong to the "pore-pollen" type. The pollen grains have a spherical or slightly spheroidal shape and their short and long diameters are 35.4—38.4 and 38.4—44.2 μ long, respectively.

Cytochemical Studies

DNA: Materials positive for Feulgen reaction are always present in the nucleus (Fig. 10). Feulgen-positive granules could not be detected in the cytoplasm of the sporogenous cell and tapetal cells. The positive stainability for Feulgen reaction of the tapetal nuclei was lost prior to the degeneration of the cells.

Polysaccharides: Lillie's method was employed for polysaccharide staining. All cells of an anther at premeiotic stage show uniformly this staining reaction. Stainability is similar in both nucleus and cytoplasm (Fig. 8). Granulous stained particles were not detected. The stainability of the tapetal cells is gradually improving after meiosis. At that time, the tapetal cells show hypertrophy and then a tendency to break down. On the other hand, the pollen mother cell shows constant stainability from premeiotic stage until separation of the microspores from the tetrad. The development of exine and formation of germinating pores follow. The latter are strongly stained (Figs. 19, 21).

The substance positive to Lillie's staining appears to be extruded into the space between the tapetal cells and the microspores (Figs. 17, 19, 20). It seems to be mucous. It is possible that it is extruded from the degenerating tapetum and is absorbed by the germinating pores and used as an energy source for the developing pollen grains. Fig. 19 shows the mucous matter adhering to the germination pores.

Prior to maturation, the pollen grains become filled with starch, while the mucous matter disappears.

Discussion

In the previous report[1]), the genus *Polygonum* was classified into five types according to the number of pollen grains in a pollen sac. Those are types with (1) 8, (2) 16, (3) 32, (4) 128 and (5) 256 pollen grains in one pollen sac. *P. persicaria* does not fall into any of those five types. It has 4 pollen grains in a sac. In the meanwhile, the present author found that *P. tenuicaule* has 64 pollen grains in one

pollen sac (Doida, unpub.). Thus, *Polygonum* can be classified into 7 types based on the number of pollen grains produced in a sac, namely types (1) with 4, (2) with 8, (3) with 16, (4) with 32, (5) with 64, (6) with 128 and (7) with 256 pollen grains per sac.

Hedberg classified the genus into 9 types on the basis of pollen morphology[6]). On the same basis, Nakai classified it into two major types[5]). Ikuse[7]) supported Nakai's opinion. One of the types is the "furrow pollen" type having three germination furrows on the exine, and the other type is the "pore pollen" type. The latter has multi-germinating pores and a reticulate pattern of the exine. This type is considered by Nakai to be characteristic of Chenopodiaceae[5]). He also stated that this type of pollen is found in *Ambryogonon*, *Tovara*, and *Persicaria*, sub-genera of genus *Polygonum*. However, Ikuse reported that Section *Didymocephalon* and *Corynbocephalon* belonging to sub-genus *Persicaria* have "furrow type" pollen. On the other hand, "furrow type" pollen appears in *Fagopyrum*, *Bistorta*, *Reynoutria*, *Aviculare*, *Pleuropterus*, *Bilderdykia* and *Pleuropteropyrum*, sub-genera of genus *Polygonum*. *P. persicaria* belongs to "pore pollen" type.

Generally speaking, "pore pollen" type appears in species producing a small number of pollen grains in a pollen sac. Types 1, 2, 3, and 4 have few pollen grains with pores, while furrow pollen type is observed in species having a comparatively large number of pollen grains, as types 4, 5, 6 and 7[1,8]).

Woodehouse[8]) tried to classify the Polygonaceae according to the shape and exine pattern of pollen grains. The process of pollen grain formation could be used in systematic or phylogenetic studies. For instance, *P. blumei* is morphologically almost identical with *P. persicaria* but they are different in the number of pollen grains produced in a pollen sac; the former species has 8 and the latter only 4 pollen grains. This difference provides a good means for distinguishing the two very similar species.

The divisions of the tapetal cell are regular in natural conditions and result in bi-nucleate cells. Tetranucleate cells resulting from successive mitotic divisions (observed by Smith[9]), Berger *et al.*[10]), etc.), restitution nuclei and polyploid nuclei following endomitosis (observed by Witkus[11]), Brown[12]), etc.) were not observed in the tapetum of this species.

The important role of tapetal cell in the normal development of sporogenous cells has been described by many authors[13,14,15]). They state that the tapetal tissue supplies nourishment to the developing sporogenous tissue. Cooper[15]) illustrated by photographs a transfer of DNA from the tapetal cells to the developing microsporocytes prior to the onset of meiosis in pollen mother cells. Regarding this point, however, Takats[16]) reported that tapetal extrusion does not occur as a regular process under normal conditions on the basis of his experiments with *Lilium longiflorum*. He also stated that the frequency of microsporocytes with chromatin globules correlates with the rate of degeneration of microsporocytes, and the frequency of microsporocytes or tapetal cells with chromatin globules is affected by handling procedures. Failure to differentiate a tapetal tissue often results in empty pollen sacs in spite of differentiation of archesporial cells in young anther primordia. Fig. 31 is a photograph of an anther of *Fagopyrum esculentum* treated with colchicine. One pollen sac has a tapetum and the other has none. The sporogenous cells develop in the former sac, but their development ceases at the stage of pollen mother cell formation in the latter (Doida, unpub.). A similar type of degeneration of the pollen mother cell appeared in *P. persicaria*. This shows indirectly that the tapetal cells have an important

role in the normal processes of microsporogenesis.

Summary

The process of pollen grain formation was observed histologically and histochemically in *P. persicaria*.

Anthers of this species have four pollen sacs, and each pollen sac has four pollen grains. The peripheral zone of a pollen sac is formed of four cell layers at premeiotic stages. The inner two cell layers disappear before the pollen matures.

Taxonomical significance of the shape and number of pollen grains is discussed. *Polygonum* can be classified into 7 types on the basis of pollen grain number per sac.

The author wishes to express his appreciation to Prof. Tamaki Shimamura of Nagoya University, and to Dr. Yô Takenaka, Head of Department of Cytogenetics, National Institute of Genetics, for their kind encouragements and advice during course of the present study. He also thanks to Dr. F. A. Lilienfeld, who was kind enough to make some corrections in the manuscript.

References

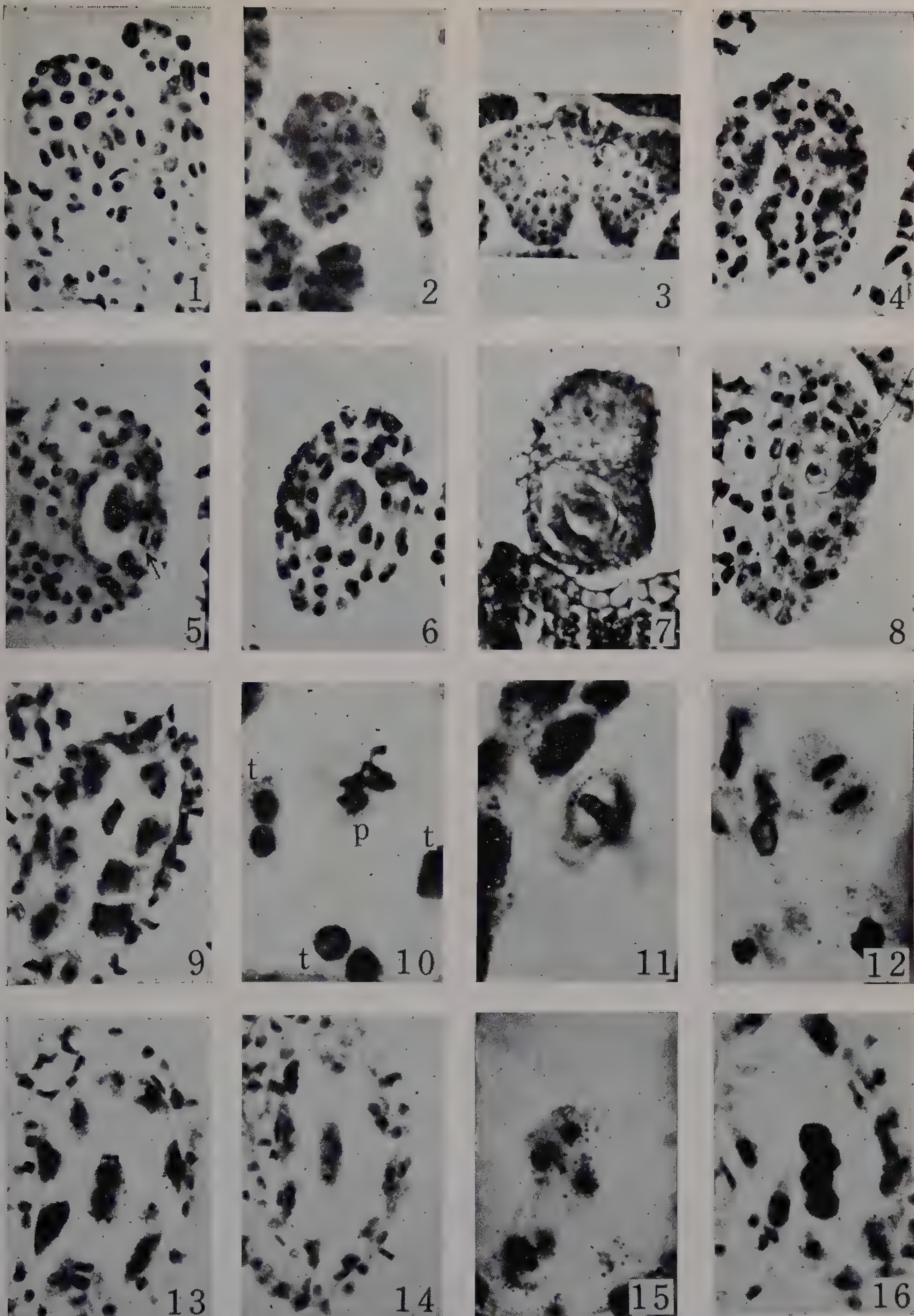
1) Doida, Y., Bot. Mag. Tokyo **70**: 31 (1957). 2) ——, Ann. Rep. Nat. Inst. Genet. (Japan) **9**: 57 (1958) 3) ——, Bot. Mag. Tokyo **72**, Suppl. Vol., p. 18 (1959). 4) Nakai, T., Bot. Mag. Tokyo **23**: 367 (1908). 5) ——, Rigakukai **24**: 289 (1926). 6) Hedberg, O., Svensk. Bot. Tidsk, **40**: 371 (1946). 7) Ikuse, M., "Pollen grains of Japan" Hirokawa Pub. Co., Tokyo (1956). 8) Wodehouse, R. P., Amer. Jour. Bot., **18**: 749 (1931). 9) Smith, F. H., ibid. **20**: 341 (1936). 10) Berger, C. A., Witkus, E. R., and Joseph, T. C., Caryologia **4**: 110 (1951). 11) Witkus, E. R., Amer. Jour. Bot., **32**: 326 (1945). 12) Brown, S. W., ibid. **36**: 703 (1949). 13) Maheshwari, P., "An introduction to the embryology of angiosperm." McGraw-Hill Book Co. Inc. London (1950). 14) Sakai, K. I., Rep. Hokkaido Agric. Exp. Stat., **43**: 1 (1949). 15) Copper, D. C., Amer. Naturalist **86**: 219 (1952). 16) Takats, S. T., Chromosoma (Berl.), **10**: 430 (1959).

摘　　要

土井田幸郎:　タデ属植物の発生学的研究 I.　ハルタデの花粉形成

ハルタデの花粉形成過程を形態形成の面から観察した．本種の葯は4室の花粉嚢をもっているが，おのおのの花粉嚢は，それぞれ4個の花粉粒を含むにすぎない．このことは葯発達の初期に(葯の横断面で)四隅に生じた1個の胞原細胞が分裂せず，そのまま花粉母細胞としての機能を有するようになるためである．すなわち花粉嚢中にただ1個の花粉母細胞が生じ，それの減数分裂で4個の花粉粒が生じるのである．

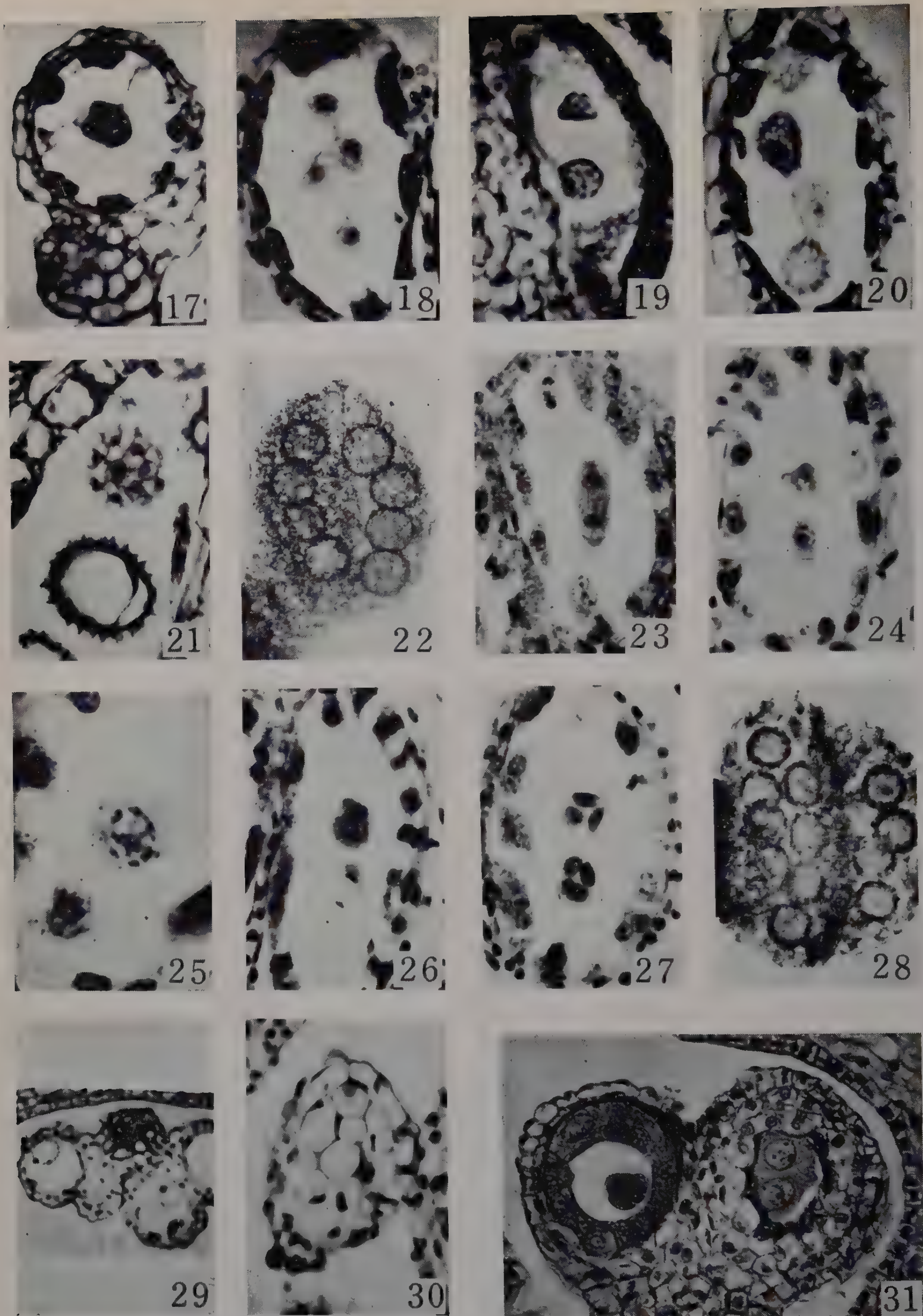
タデ属植物の花粉形成過程の研究により，花粉嚢あたり形成される花粉粒の数をもとに本属を5型に分けたが，本研究によりハルタデはそのいずれにも属さないことが解った．——ハルトラノオは他の1型を示す——以上のことより本属は7型に分けうることになる．すなわち，一花粉嚢あたり 4, 8, 16, 32, 64, 128 および 256 の花粉数を有する場合である．一方花粉形態をもとに，本属は2型に大別できるが，この点と花粉形成の7型との間の関係についても論じた．(名古屋大学理学部生物学教室)



Process of microsporogenesis in *P. pericsaria*. H, F, L and A in parentheses show the method used for staining; H: Hematoxylin staining, F: Feulgen reaction, L: Lillie's method for polysaccharides, and A: aceto-carmine.

Fig. 1. Young anther primordium (H). Fig. 2. The same stage as in Fig. 1 (H). Fig. 3. Cross-section through a young anther; the shape is four-lobed (H). Fig. 4. A differentiated archesporial cell (H). Fig. 5. Peripheral zone of anther consists of three cell layers. Arrow shows a mitotic division in a cell of the transitional layer (H). Fig. 6. Four layers are formed at the periphery (H). Fig. 7. Premeiotic stage (L). Fig. 8. Premeiotic stage (H). Fig. 9. Binucleated tapetal cell (H). Figs. 10–12. Meiosis. Fig. 10. The PMC (p) in meiosis and binucleated tapetal cells (t) (F). Fig. 11. Metaphase I (H). Fig. 12. Telophase I (H). Fig. 13. Interphase I (H). Fig. 14. A linear tetrad (H). Fig. 15. Y-shaped tetrad (H). Fig. 16. Linear arrangement of microspores (H).

Y. DOIDA: Developmental Studies in the Genus *Polygonum*. 1. Microsporogenesis of *Polygonum persicaria* L.



Process of microsporogenesis in *P. persicaria*. H, F, L and A in parentheses show the method used for staining; H: Hematoxylin staining, F: Feulgen reaction, L: Lillie's method for polysaccharides, and A: aceto-carmine.

Fig. 17. Cross-section of anther having a tetrad (L). Figs. 18–20. Development of four microspores (L). Fig. 21. Mature pollen grains with strongly stained germinating pores (L). Fig. 22. Anther having four pollen grains in a each pollen sac (A). Figs. 23–28. Aberrant pollen grain formation (H). Fig. 23. Mitotic division of the archesporial cell. Fig. 24. Two PMCs. Fig. 25. Prophase 1 in two PMCs. Fig. 26. Telophase II. Fig. 27. Two tetrads. Fig. 28. One pollen sac with 8 and the other with 4 pollen grains (A). Fig. 29. One of four pollen sacs degenerates (H). Fig. 30. Empty pollen sac (H). Fig. 31. An anther of *Fagopyrum esculentum* treated with colchicine. Left pollen sac has a tapetum but the right one has none. The development of the sporogenous cell proceeds in the left sac although it is abnormal, but ceases in the right one.

Y. DOIDA: Developmental Studies in the Genus *Polygonum*. 1. Microsporogenesis of *Polygonum persicaria* L.

Bot. Mag. Tokyo, **73**: 283—287 (July 25, 1960)

Studies on the Growth of Fruit Body of Fungi II.* Activity and Stability of the Growth Hormone in the Fruit Body of *Agaricus bisporus* (Lange) Sing.**

by Hiroshi HAGIMOTO*** and Michio KONISHI***

Received December 22, 1959

Existence of a special growth hormone active to the growth (pileus expansion and stipe elongation) of fruit body was demonstrated in *Coprinus lagopus*[1]), in *Collybia velutipes*[2]) or in *Agaricus bisporus*[3,4]) and other Basidiomycetes[4]). The growth of stipe decreased by removal of pileus at early stage, or the stipe curved by half or uneven removal of pileus or gills, by half-insertion of mica plate, or by splitting the stipe. On the other hand, Jeffreys and Greulach obtained no significant curvatures on *Coprinus sterquilinus* by unilateral placing of agar blocks containing fruit body diffusates of the same species on the stipe stump[5]). Evidences decisive to the existence of the growth hormone and its partial isolation by diffusion and extraction method are described in the present paper.

Materials and Methods

The white strain and sometimes snow white strain of commercial mushroom, *Agaricus bisporus* (Lange) Sing. and some other Basidiomycetes were used. In commercial mushrooms the fruit bodies grown on new flats were used for the experiments; the mushrooms grown on the old flats often showed growth abnormality such as onion like form. Other mushrooms than *A. bisporus* were collected in the fields at our vicinity.

An "*Agaricus test*", corresponding to the "*Avena test*" in higher plants, was adopted, as has already been suggested in a previous paper[4]). At first, bioassay was performed at the Morimoto Mushroom Nursery as in the previous experiment, later on, in the Phytotrone room regulated at $15\pm1°$ and R. H. ca. 90%.

Procedure of the "*Agaricus test*": Fruit bodies which have reached about 30 mm. in length, standing vertically and growing solitary, are selected. When growing crowded, all fruit bodies but one to be used were removed. With a razor blade a pair of opposit parts of pileus and stipe from a plant on the flat was cut off along parallel plains (Figs. 1A and A′ in the previous report[4])). From thus resulted T-shape plant having wings of pilei on both right and left side the whole gills were removed with the care not to injure the stipe (Fig. 1C in the previous report[4])). On one of the lower surface of pileus wings thus treated, materials to be tested, such as agar blocks containing test substance, and on the other, plain agar blocks as the control were attached (Fig. 1A). All the manipulations were performed under artificial light and thereafter the plants were allowed to grow in the nursery house or phytotrone, having sometimes weak illumination. In our mushroom, the stipe curvatures in both intact and completely gill-free fruit bodies were not caused by unilateral illumination of light, as has already been observed by Buller[6]). The re-

* This work was supported by a grant from the Rockefeller Foundation.

** This paper was presented partially on October 25th, 1958 to the 23rd annual meeting of the Botanical Society of Japan, held at Fukuoka, Japan and on September 4th, 1959 to the 24th annual meeting of the Botanical Society of Japan, held at Sendai, Japan.

*** Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

sultant stipe curvature, which showed the existence of growth hormone, became visible within 2 or 3 days of the treatment (Fig. 1B). The agar blocks attached to the lower surfaces of pileus wings usually dried up within 24 hours after the treatment (Photo. 1 and 2). The curvature was recorded on the 3rd or 4th day after the treatment. Excess of dryness in the relative humidity far less than 90% which is favorable for the growth is to be avoided. More than 12 fruit bodies were used for one test. Agar blocks used for the bioassay were 2.5% gel and about 10×10×3mm. in size. The blocks were stored in 60% ethanol solution, washed overnight with distilled water at about 5° before use. This test may be of little value from a quantitative point of view but is enough to prove the existence of activity of test substance.

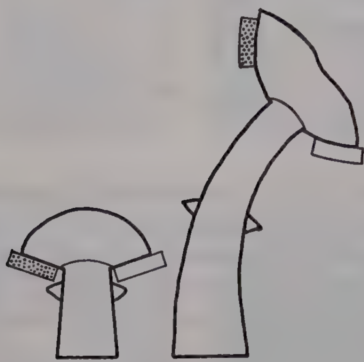


Fig. 1. Diagram showing the procedure of the "*Agaricus* test". ×⅔. A: Attaching of agar blocks on the lower surface of the pileus from which the whole gill part of pre-treated fruit body (see the previous report[4]) was removed; the dotted agar block contains the test substance. B: Resultant curvature caused by the active growth hormone.

Purified petroleum ether, benzene, ether, acetone and ethanol were used as the solvents for the extraction. Gills detached from the pileus or occasionally pilei with gills or whole plants were used for the extraction. The extracts resolved in an adequate amount of distilled water and diffused in agar blocks were tested.

Results and Discussion

A. Effect of gills on the growth of fruit body.

A part of pileus with gills or a piece of gill part was pasted with agar gel on the lower surface of pileus of gill-free test plant. Negative curvature and much expansion of the pileus wing at gill attached side were observed as in Hawker's and Urayama's experiment[2,3]). The intense expansion of pileus wing due to gill action was, in general, observed at 30 mm. or less long fruit body. It is to be ascertained whether one and the same hormone was active in both of the phenomena, i.e. the pileus expansion and the stipe elongation. Gills from the snow white strain also gave the same result on the white strain. The converse held also true. Sometimes mycelia of unknown nature were observed in the agar block with excised gills. For the purpose of a convincing demonstration, gill parts taken from a 30 mm. long fruit body were placed on agar blocks (10×10×3 mm.) in a moist Petri dish and kept for 24 hours at about 5° in order to check mycelial development. These agar blocks containing gill diffusate were tested. During the test, the agar blocks were changed twice at interval of 24 hours or were removed at 24th hour after the treatment. The gill diffusate itself is thus active to the growth of the fruit body.

There may be another possibility remaining, that is, the substance occurring in gills may not be true hormone itself but it generates or activates the hormone, or changes in itself to the hormone on the way of transportation. A small piece of gill block was attached with agar gel directly to a cut vertical surface of growth zone of a plant prepared for the curvature test (Fig. 2A, B). Bending-off of the stipe from the applied side was observed though the curvature was not so strong (Fig. 2C).

A direct hormonal stimulation by the substance is highly probable.

Not only a piece of gills but a piece of pileus-flesh or even of stipe were also active to the *Agaricus* test, probably showing an existence of transporting growth hormone in these tissues.

B. Extraction of the growth hormone.

a) Extraction with water. The hormone is to be water soluble since it was diffusible into agar gel. Twelve grams of fresh gills from about 30 mm. long fruit bodies were soaked in 25 m*l*. distilled water containing 12 agar blocks. After keeping at about 0° for two days, the agar blocks were tested. The resultant stipe curvatures clearly showed that the hormone diffused into the water out of the tissue. Extraction of gills with boiling water gave also successful result: thirty grams of fresh gills were put into 100 m*l*. of boiling distilled water and this was immediately placed at about 5° in an electric refrigerator. After two days the water solution was filtered and condensed to 10 m*l*. with rotary flash evaporator under a reduced pressure of about 20 mm. Hg. Agar blocks soaked in the condensed solution and kept overnight at 5° caused clearly negative curvatures. Fresh gills were enveloped with cellophane and steeped in distilled water at 5° for 2 days, and it was found that the active substance was diffusible through cellophane membrane. The hormone would be of small molecular size.

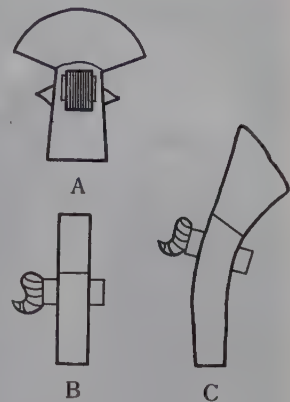


Fig. 2. Curvature caused by direct attachment of small part of living gills (lined square) with agar blocks on the growth zone of stipe. $\times 2/3$. A: Front view; On the opposite side a plain agar block is placed. B: Side view. C: Curvature caused by the treatment (side view).

b) Extraction with organic solvents. The growth hormone was tried to extract from fresh gills (30 g.) with petroleum ether (100 m*l*.). After removal of the solvent under reduced pressure, the residue, after adding 10 m*l*. distilled water and agar blocks, was tested. In such manner, extraction was performed with benzene, ether, acetone, and ethanol. In order to exclude water from the fresh gills, each extract was re-treated with same but completely dehydrated solvents. Ether, acetone and ethanol extracts showed positive result; the ethanol extract, for example, caused negative stipe bending and positive pileus expansion to 19 fruit bodies out of 21 used.

In another series of experiment extractions were started from the ethanol extract of fresh gills: 30 g. fresh gills were soaked into 100 m*l*. boiling ethanol and kept at 0° for 2 days. The crude extract thus obtained was dealt with 100 m*l*. petroleum ether, benzene, acetone or ether. After removal of the solvents under a reduced pressure, each residue was dissolved in 10 m*l*. of distilled water and tested. Fractions soluble to ether, acetone and ethanol, and those insoluble to the organic solvents used were active (Photo. 1). Further purification of the hormone is now in progress.

C. Stability of the growth hormone.

Gills or water solution of ethanol extract of gills were heated on boiling water bath for an hour in Erlenmyer's flask with a reflux condenser. The *Agaricus* test gave positive results, i.e., the stipe curved and the pileus expanded (Photo. 2). Some activity was maintained even when the extract was heated for an hour with N–HCl or –NaOH and was neutralized by N–NaOH and N–HCl.

Photo. 1. Curvature caused by the ether extract of gills. At the right side an agar block containing the ether extract (arrow). ×ca. 1.

Photo. 2. Curvatures caused by attaching an agar block with a small part of gills previously heated for an hour at 100° to the lower surface of pileus on the left side; Plain agar block on the right side. Greater expansions occur on the side with heated gills on each pileus. ×ca. ⅔.

Water solution of the hormone can be retained more than 4 weeks at −5° without any loss of activity. No loss was also observed when the hormone dryid for five days on paper exposed to room light and temperature. But when water solution of the extract is kept unsterilized at room temperature for few days, the activity is not only lost but sometimes positive (and zero) curvature of stipe is observed. This was always the case in various concentrations tested. It may be thus evident that the positive curvature was never caused by an excess of the hormone but presumably due to microbial destruction of the hormone and production of some inhibitory substance.

D. Relation of the growth hormone to auxin.

The above extracts active to the growth of fruit body showed no activity to the *Avena* test, although a considerable amount of auxin, mostly acid and bound form of β-indole-3-acetic acid (IAA), has been detected in fresh gills, gill-free portion of pileus or in stipes[7]). It is probable that IAA was destroyed during the extraction with heated ethanol and only the mushroom hormone was remained.

IAA in agar blocks at concentrations from 0.1 to 1000 ppm. at intervals of ten times gave also no curvature in *Agaricus* test. Some investigators also reported that fruit body of fungi did not react to external applications of IAA[3,5]). The mushroom growth hormone is thus, as has already been suggested by Urayama, to be different from IAA and may have no relation to the growth of higher plants. Moreover, acid- and alkali-stability of the hormone supports this view, i.e., IAA must be destroyed by heating in N-HCl. The results, however, do not mean that IAA is unnecessary for the growth of fruit body. Studies on IAA in the fruit body will be presented in near future[7]).

E. The active substance or substances obtained from other Basidiomycetes than *A.*

bisporus.

The authors have already reported that the growth of fruit bodies of *Coprinus* and of other fungi seems to be also regulated by growth hormone[4]). New experiments on ethanol extracts obtained from pilei of *Coprinus macrorhizus* f. *microsporus* (*C. fimetarius*), *Hypholoma fasciculare* and from gills of *Armillaria matsutake* were also positive, i.e., negative stipe curvatures and much expansion of the pileus wing at the extract given side were observed on the *Agaricus* test. It is yet unknown whether the growth hormone of these field mushrooms is chemically identical with that of commercial mushroom.

Summary

1) Existence of growth hormone or hormones promoting stipe elongation and pileus expansion of *Agaricus bisporus* was ascertained directly by diffusion and extraction method.

2) The hormone can be transferred from gills into agar gel and is dialysable from a water solution through cellophane membrane.

3) The hormone is soluble in ether, acetone, ethanol and water, and insoluble in petroleum ether and benzene.

4) The hormone is heat-stable and is not inactivated when boiled for one hour even in N–HCl or –NaOH.

5) The hormone is not effective to the *Avena* test; IAA gave no curvature in the *Agaricus* test. The hormone is not to be identical with IAA or its allies.

6) Extracts obtained from *Coprinus macrorhizus* f. *microsporus*, *Hypholoma fasciculare* and *Armillaria matsutake* were active to the growth of fruit body of *A. bisporus.*

The authors wish to express their sincere thanks to Prof. S. Imamura and Assoc. Prof. M. Hamada for their interests during the work.

References

1) Borriss, H., Planta **22**: 28 (1934), 2) Hawker, L. E., Physiology of Fungi, University of London Press (1950). 3) Urayama, T., Bot. Mag. Tokyo **69**: 298 (1956). 4) Hagimoto, H., and Konishi, M., Bot. Mag. Tokyo **72**: 359 (1959). 5) Jeffereys, D. B., and Greulach, V. A., Jour. Elisha Mitchell Sci. Soc. **72**: 153 (1956). 6) Buller, H. R., Ann. Bot. **19**: 427 (1905)· 7) Konishi, M., and Hagimoto, H., Plant and Cell Physiol. (in press).

摘　　要

萩本　宏·小西通夫: 菌類子実体の生長に関する研究 II. ツクリタケ (西洋マツタケ) 子実体生長ホルモンの若干の性質

1. 著者等は子実体生長ホルモンが存在することをすでにツクリタケその他の担子菌子実体を用いて間接に証明した.この論文では子実体生長ホルモンの存在を拡散や抽出操作により一層確実に証明し, さらにホルモンの単離のための予備実験として,若干の性質を明らかにした. 2. この生長ホルモンはヒダから寒天塊に容易に拡散し, またセロファン透析が可能である. 3. この生長ホルモンはエーテル, アセトン, エタノールおよび水に可溶, 石油エーテルおよびベンゼンに不溶である. 4. この生長ホルモンは熱のみならず, 酸およびアルカリにも安定である. 5. この生長ホルモンは *Avena* テストにかからない. 逆に 0.1, 1, 10, 100 および 1000 ppm の濃度のインドール酢酸 (IAA) は *Agaricus* テストにかからない. したがって子実体生長ホルモンは IAA あるいはその類縁化合物ではないと考えられる. 6. ウシグソヒトヨ, ニガクリタケおよびマツタケのカサあるいはヒダからの抽出物も *Agaricus* テストにかかった. 子実体生長ホルモンの存在は普遍的であると考える.

本実験の *Agaricus* テストの大部分は森本養菌園 (京都市伏見区桃山) で行なった. 園主森本彰氏に深く感謝の意を表する. (京都大学農学部応用植物学研究室)

Bot. Mag. Tokyo, **73**: 288—294 (July 25, 1960)

The Genus Scirpus in the Hawaiian Islands

by Tetsuo KOYAMA* and Benjamin C. STONE**

Received January 9, 1960

The purpose of this paper is to reinvestigate all species of the genus *Scirpus* (Cyperaceae) known from the Hawaiian Islands. There has not been much work on *Scirpus* in Hawaii. Only two species of the genus were known to Hillebrand in his 'Flora of the Hawaiian Islands' (1888). Kükenthal added a new species from Kauai based on a collection by J. F. Rock in 1909. More recently, Beetle (1941) reported some other specimens and revised a few names concerned. In general, the names of the species of *Scirpus* now seem to be rather well understood. However, since these species very often show a tendency of interregional or intercontinental occurrence, the comparison of Hawaiian specimens with continental specimens, and a more satisfactory solution of the names involved has seemed necessary.

Key to the Hawaiian species of Scirpus

A. Inflorescence terminal or nearly so, subtended by leaf-like bracts; leaves with a normal gramineous blade.......................... 1. *S. maritimus*
A. Inflorescence pseudo-lateral with an erect culm-like bract; all leaves reduced to bladeless sheaths.
  B. Inflorescence of a contracted head; plants of medium size, culms less than 6 dm. tall... 2. *S. juncoides*
    C. Spikelets oblong-cylindric, 9–20 mm. long, 3.5–5 mm. broad; floral scales obtuse or retuse at apex................... 2a. var. *Ohwianus*
    C. Spikelets ovate-oblong, 8–15 mm. long, 3–3.5 mm. broad; floral scales contracted and mucronate at apex................ 2b. var. *Rockii*
  B. Inflorescence of an open panicle with elongate rays; large plants with culms more than 1 m. tall.
    C. Perianth bristles 5–6 per achene, retrorsely scabrous with acute spinules ....3. *S. lacustris*
    C. Perianth bristles 2–4 per achene, plumose.
      D. Culms trigonal......................... 4a. *S. californicus*
      D. Culms terete.......................... 4b. var. *tereticulmis*

1. **Scirpus** (Bolboschoenus) **maritimus** Linn., Spec. Pl. ed. 1, 51. 1753.
var. **paludosus** (A. Nelson) Kükenthal, Fedde Rep. Sp. Nov. **23**: 200. 1926.

Sc. maritimus L. var. *digynus* Hillebr., Fl. Haw. Isls. 475. 1888. not of Boeckeler, 1870.

Sc. paludosus A. Nelson in Bull. Torr. Bot. Club **26**: 5. 1899.—Beetle, in North Amer. Flora **18** (8): 483. 1947.—Fernald in Gray's Man. Bot. ed. 8, 272, incl. var. *atlanticus* Fern. & fig. 442. 1950.

Sc. paludosus A. Nels. var. *atlanticus* Fern., Rhodora **45**: 291. 1943.

Sc. paludosus A. Nels. var. *digynus* (Hillebr.) Beetle, Leafl. West. Bot. **4**: 47. 1944.

* Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

** Botany Department, University of Hawaii. Present address: Department of Botany, Smithsonian Institution, Washington 25, D. C., U.S.A.

OAHU: Koko Head, Maunalua, 1924 (J. A. Harris 242153!), Waialae, 1929, (E. L. Caum s.n.!), Kapiolani Park, Honolulu, 1923 (G. P. Wilder 50!), (G. C. Munro 1!), Heeia Bridge (F. E. Egler 37–137!), Waikiki, 1930 (Wilder s.n.!), Waikiki, 1916 (A. S. Hitchcock 13808!), Kailua, 1929 (H.St. John 10044!), Kailua stream, 1920 (Stokes s.n.!), Kipapa gulch, Waipahu, 1932 (E.Y. Hosaka 759!), Honolulu, 1919 (Stokes s.n.!), Ala Moana marsh, 1939, (M. C. Neal 1192!), Mt. Tantalus, Honolulu, 1953 (G. Pearsall s.n.!), sine loco speciali (H. Mann & Wm. Brigham 230!, O. Degener 8995!).

MOLOKAI: Puko'o (C.N. Forbes 408–Mo.!).

NIIHAU: Southern end of island, 1912 (J. F. G. Stokes s.n.!).*

Distrib. Southern Canada, United States, West Indies, Argentina, Australia.

Hawaiian name: '*kaluha*'**.

The difference between *S. paludosus* of North America and *S. maritimus* of Europe is very slight. Fernald and Beetle, after all, explained it as, in the shape of the achenes, that the base of the achenes is gradually rounded in *S. maritimus*, while it becomes cuneate in *S. paludosus*; and the achenes are lenticular in *S. paludosus*, but compressed or sharply trigonous in *S. maritimus*. Further, according to Fernald, *S. paludosus* can be distinguished by the color of its spikelets, whitish brown to drab, rather than cantaneous or fuscous. But, as Koyama has observed in the Japanese representative of the *S. maritimus* group (cf. Koyama in Journ. Fac. Sci. Univ. Tokyo 3, **7**: 333. 1958), there is a very wide deviation in the size and shape of the achenes in many species of the group of Bolboschoenus. So far as we observed, specimens of *S. paludosus* are also quite variable, characterized by the much lighter color of the scales, somewhat obtusely-angled achenes, and less acute tips of the achenes. Therefore, in regarding *S. paludosus* to be a variety of the wide-spread *S. maritimus*, we quite agree with Kükenthal, who stated "Die Variation zeichnet sich durch hellere Deckschuppen, linsenförmige Frucht und zwei Narben aus."

2. **Scirpus** (Actaeogeton) **juncoides** Roxburgh. (Hort. Beng. 81. 1814. nom. nud.), Fl. Ind. 1: 228. 1820.—T. Koyama, Journ. Fac. Sic. Univ. Tokyo 3, **7** (6): 310–315, f. 9. 1958.

For the detailed synonymy, see Koyama l. c.

Distrib. sp. Japan, Ryukyus, Formosa, China, India, Malaysia, Micronesia, Australia.

2a. var. **Ohwianus** (T. Koyama) T. Koyama, l. c. 311, f. 9, b–g. 1958.

Sc. Ohwianus T. Koyama, Bot. Mag. Tokyo **69**: 212, f. 4, 1956.

KAUAI: Waimea, 23 Oct. 1895 (A. A. Heller 2891!).

Distrib. Japan, Ryukyus, Formosa, Philippines (Luzon).

A new record for the Hawaiian Islands. This taxon is characterized by long thick spikelets, and thick culms up to 70 cm. tall and 7 mm. thick. It is the largest of all variants of the polymorphous *S. juncoides*. The only collection from Hawaii is Heller's, which has the spikelets up to 23 mm. long with more than 13 rows of scales. Its achenes are broadly obovate to almost obdeltoid, with 4–6 perianth bristles of various length, four of which are about equal in length to the achene. Its scales are obtuse to slightly retuse at the tip. Thus the specimens appear to be very different from the common Hawaiian race, var. *Rockii*, in the characters of the achenes and floral scales, and well agrees with var. *Ohwianus* of Japan. It is inter-

* All specimens cited here are kept in the herbarium of the Bishop Museum (BISH).

** Hawaiian names cited here have been given by Mrs. Mary K. Pukui of the Bishop Museum, Honolulu.

esting that we have a few other examples of species of Cyperaceae with a Hawaiian-Japanese link. They are the *Carex wahuensis-Boottiana* complex, and the *Carex brunnea-Meyenii* complex. Var. *Ohwianus* also seems to come under this geographical category.

There is another interesting aspect, however. According to Heller (Minnesota Bot. Studies **9**: 803. 1897), "at Waimea, Kauai, a Scirpus was collected in taro ponds (viz. at relatively low elevations where Colocasia was grown–TK & BCS) which has not yet been satisfactorily placed. It is probably an introduced plant, allied to *Scirpus debilis*". If Heller's supposition is correct, that it was introduced, it is likely that it was accidentally (or purposely ?) brought in with rice, which was to be grown in Kauai for them then by recent Japanese immigrants. The seeds of this *Scirpus*, perhaps, could have become intermixed with rice (either seeds or wet cuttings), and thus, after their introduction to Hawaii, could have spread.

Finally, it does not seem too conjectural an idea to suggest that perhaps the var. *Rockii*, found not only at the highest elevations on Kauai, at 4000 feet, but also (at Kahili) in a bog at 1500 ft., might be a race of var. *Ohwianus* which developed after the introduction of var. *Ohwianus*. Alternatively, there is the possibility that var. *Rockii*, as such, may yet be discovered somewhere in Japan or nearby. Or lastly, that what has been assumed all along, that var. *Rockii* is a truly indigenous plant which arrived in Hawaii long before human inhabitants, is true. At present, there does not seem to be enough information to state definitely which of these three possibilities is most likely. However, it is almost certain that var. *Ohwianus* is of recent introduction, perhaps associated with the date of the early immigrations of Japanese to Hawaii, before 1890.

2b. var. **Rockii** (Kükenthal) T. Koyama & B. C. Stone, stat. nov. ex isotypo!
Sc. Rockii Kükenth., Fedde, Rep. Sp. Nov. **16**: 432. 1920.

KAUAI: Kokee, Lehua Makanoe bog (O. Degener, I. Degener, H. W. Hansen & R. K. Hanner 23943 ! in 1955), trail from Kokee-Mohihi road to Lehua Makanoe bog, 1938 (L. Cranwell, O. Selling, & C. Skottsberg 2933!), between Kokee and Kilohana, 1922, (Skottsberg 980!), Alakai swamp, with *Oreobolus* and *Drosera*, 1952 (O. Degener 21733!), Alakai swamp, Kokee, 1956 (B. C. Stone 1528!), ibid. (I. E. Lane 56–612);—Hanalei-Kalihikai power line trail, 600–1600 ft., 1931 (H. St. John *et al.* 10970!), Halemanu, 1200 m, 1927 (L. H. McDaniels 749!), Kaholuamano, 4500 ft., 1909 (J. F. Rock 5141! isotype of *S. Rockii*), Wahiawa bog, base of Pu'u Kahili, 1500 ft., 1957 (I. E. Lane 57–2!).

Distrib. Endemic to Kauai.

Hawaiian name '*kaluha*'.

This is a Hawaiian population of *S. juncoides* Roxb., a species which is widespread over the Asian temperate and tropical regions. When Kükenthal described *S. Rockii*, he found its affinity with the North American *S. Torreyi*, a species having triangular culms. But in comparing *S. Rockii* with *S. juncoides*, especially the var. *Ohwianus*, it is seen to approach the latter in every character except the scale-apex. In var. *Rockii* the apex of the scales is always tipped with a short pointed mucro of varying length, while in var. *Ohwianus*, the scale apex is retuse with a very short mucro in the notch, not exceeding the upper margin of the scale. The perianth bristles are relatively shorter in var. *Rockii* and the achenes are slightly narrower. We are thus inclined to reinterpret *S. Rokii* as a variety of *S. juncoides*. Further remarks on the relationship to var. *Ohwianus* are given above.

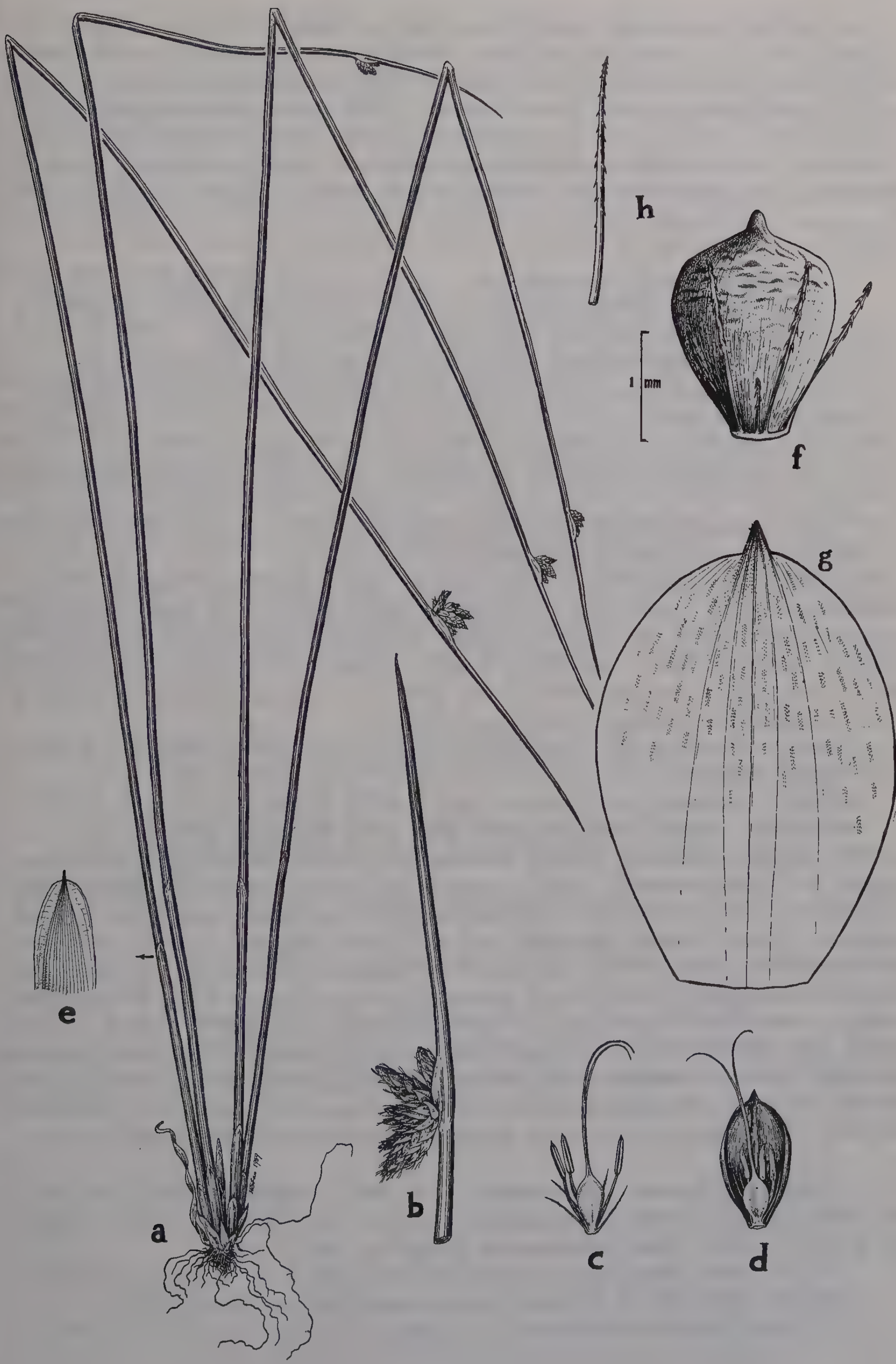


Fig. 1. *Scirpus juncoides* var. *Rockii* (Kükenthal) T. Koyama & Stone, stat. nov.
a–e, h; from B. C. Stone 1528, Alakai swamp, Kauai.
f–g; from I. E. Lane 57–2, Wahiawa bog, Kauai.
a. Habit, ×1/2 b. Inflorescence, ×1. c. Flower, withscaleremoved, ×5.
d. Same, with scale, ×5. e. Tip of leafless culm, ×3. f. Achene, ×15.
g. Scale, ×15. h. Bristle, ×20.

As far as is known, this plant occurs only on Kauai. Of all these specimens, probably only two groups may be made, corresponding to two more or less similar boggy areas, first at Kokee and at about 4000 ft. elevation, the second near the base of Pu'u (Mt.) Kahili at about 1500 ft. elevation. The Hanalei-Kalihikai trail is another low-level area. Wahiawa bog is the name for the bog at the base of Kahili.

Certain plants of these bogs fall into a geographic group included in a northern-hemisphere, temperate zone area. Such plants as *Drosera anglica*, the genus *Viola*, etc., occur in such areas.

3. **Scirpus** (Lacustres) **lacustris** Linn., Spec. Pl. ed. 1, 48. 1753.
subsp. **glaucus** (Smith) Hartman, Svensk. Og. Norsk Exc. Fl. 10. 1846.--T. Koyama, Journ. Fac. Sci. Univ. Tokyo 3, **7** (6): 321, 1958.
forma **luxurians** (Miquel) T.Koyama, 1. c. 324, f. 9, d–e, 1958.

Sc. Tabernaemontani Gmel. forma *luxurians* Miquel, Ann. Mus. Bot. Lugd.-Batav. **2**: 143. 1856.

'*Sc. lacustris* Linn.'; Hillebr. Fl. Haw. Isls. 475. 1888.

Sc. validus Vahl, Enum. Pl. 2: 268. 1806.—Neal, In Gardens of Hawaii, 80, 1948.

Sc. Tabernaemontani Gmel. forma *australis* Ohwi, Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B, **18** (1): 121. 1944.

OAHU: Kaau crater, Palolo valley, Koolau Mts., 1700 ft. alt., 1942 (H. St. John 20319!), ibid. (F. E. Egler 37–318!), Nunanu valley, 1895 (A. A. Heller 2047! cited in Minn. Bot. Stud. **9**: 803, 1897), Kapiolani Park, Honolulu, 1948 (G. C. Munro s.n.!), Hau'ula, northern Oahu, 1929 (E. H. Bryan, Jr. 695!), Ala Moana, Honolulu, 1939 (M. C. Neal 1193!), Hau'ula, 1916 (Forbes 2364. O.!), sine loco speciali (H. Mann & Wm. Brigham 25!, Galathea-Expedition in 1845–7!).

KAUAI: Wahiawa bog, Pu'u Kahili, 2100 ft. alt., 1933 (H. St. John & F. R. Fosberg 13556!).

NIIHAU: southern end, in ponds. 1912 (J. F. G. Stokes s.n.!).

MOLOKAI: Puko'o, along the shore, 1916 (A. S. Hitchcock 15112!).

For detailed synonymy see T. Koyama 1. c. 1958. According to this investigation, *S. Tabernaemontani* (=ssp. *glaucus*) can not be specifically distinct from *S. lacustris* s.str. of Europe. The distribution of ssp. *glaucus* covers the Eurasian continent, North America, Malaysia, and the Pacific Islands. In this large area are found two forms, viz. f. *glaucus* (=f. *normalis* Miquel), and f. *luxurians*, which are somewhat geographically separated. Forma *glaucus* is the northern population, chiefly characterized by the spikelets always in groups of 2 to 4, and the scales dark red-brown with a broad fimbriate upper margin. Forma *luxurians*, on the other hand, is a southern population, of which spikelets are as a rule solitary, and the scales are ferrugineous-brown, with a narrower upper margin not exceeding the awn. The latter form occurs in southern Asia and the Pacific Islands.

4. **Scirpus** (Littorales) **californicus** (C. A. Mey.) Steudel, Nomencl. Bot. ed. 2, 538. 1841—Abrams, Illustr. Fl. Pacific St. **1**: 274, f. 459. 1923—Beetle in North Amer. Fl. **18** (8): 504. 1947—Neal in Gardens of Hawaii 80. 1948.

Sc. riparius Presl, Reliq. Haenk. **1**: 193. 1828—C. B. Clarke in Engl., Bot. Jahrb. **30**, Beibl. Nr. 68, 36. 1901—non Persoon 1805, nec Poiret 1817.

Elytrospermum californicum C. A. Meyer, in Mém. Sav. Etr. Pétersb. **1**: 200, t. 2. 1831.

Malacochaete Tatora Nees et Meyen ex Nees (in Linnaea **9**: 292. 1834. nom. nud.), Nov. Act. Nat. Curio. **19**, Suppl. **1**: 90. 1843.

Sc. Tatora (Nees et Mey.) Kunth, Enum. Pl. **2**: 166. 1837.

Schoenoplectus riparius (Presl), *Tatora* (Nees et Mey.), et *californicus* (C. A. Mey.) Palla, in Engl. Bot. Jahrb. **10**: 299. 1888.

Sc. californicus Steud. var. *Tatora* (Kunth) Barros, Darwiniana **6**: 126. 1942.

OAHU: Kailua, Kaalepulu stream, 1920 (J. F. G. Stokes s.n.!).

MOLOKAI: Puko'o, 1912 (C. N. Forbes 9. Mo.!)

HAWAII: Hamakua-Waikoloa, 2500 ft., 1942 (E. Y. Hosaka 2659!). Distrib. North and South America.

Hawaiian Name: '*neki*' (Kauai), '*aka'akai*' (Oahu), '*nanaku*' (Hawaii).

The Hawaiian plants of this species may perhaps be introduced. Beetle (1941, 1949) is of the opinion that *S. californicus* and *S. Tatora* are distinct species. But we regard these two rather variable plants as conspecific. They have a distribution in the American continent, both North and South. Several varieties have been described both under *S. californicus* and under *S. Tatora*, and all those of *S. Tatora* were transferred to *S. californicus* by Beetle in 1949. One of them, *S. californicus* var. *Chamissoi* (Nees) Beetle, from Brazil, has not been examined by us, but the other three varieties, viz. vars. *tereticulmis*, *paschalis*, and *spoliatus*, are here treated as being the same taxon, which is characterized by its terete culms. The angularity of culms is in some species rather variable. Intermediate forms between angular and terete culms can be seen in such species as *S. juncoides*, *S. littoralis*, and the present one, just as C. B. Clarke stated when he reduced *S. tereticulmis* Steud. to *S. riparius* (=*S. californicus*) as "Var. mihi trivialis, in S. riparium typicum sensim transiens." (l. c. 1901).

4b. var. **tereticulmis** (Steud.) Beetle, in Madroño **6**: 48. 1941.

Sc. riparius Presl var. *tereticulmis* (Steud.) C. B. Clarke, in Engl., Bot. Jahrb. **30**, Beibl. Nr. 68, 36. 1901.

Sc. tereticulmis Steudel, Syn. Pl. Glumac. **2**: 85. 1855.

Sc. pseudotriqueter Steudel, l. c. **2**: 86, 1855, et in Lechler. Berber. Amer. Centr. 50. 1857.

Sc. riparius Presl var. *paschalis* Kükenthal, Fedde Rep. Sp. Nov. **16**: 432. 1920.

Sc. californicus Steud. var. *paschalis* (Kük.) Beetle, Amer. Midl. Natur. **41** (2): 459. 1949.

Sc. californicus Steud. var. *spoliatus* Barros, Lilloa **1**: 69. 1937.

KAUAI: Kokee stream, 1922 (C. Skotssberg 987!), Kokee, 3500 ft. alt., 1929, (Smith, Whiting & Neal s.n.!), Waineke swamp, Waimea drainage basin, 1917 (Forbes 852. K.!).

Distrib. South America.

References

Beetle, A. A. Specific and varietal transfers in Cyperaceae. Leafl. West. Bot. **4**: 44-47. 1944. —Heller, A. A. Observations on the Ferns and Flowering Plants of the Hawaiian Islands. Minnesota Botanical Studies **9**: 760-922, illustr. 1897.—Hillebrand, W. Flora of the Hawaiian Islands. Heidelberg. 1888.—Koyama, T. Taxonomic study of the genus Scirpus Linné. Journ. Fac. Sci. Univ. Tokyo, 3, **7** (6): 271-366, illustr. 1958.—Kükenthal, G. Cyperaceae novae V. Fedde, Repert. Sp. Nov. **16**: 430-435. 1920.—Neal, Marie C. In Gardens of Hawaii. Honolulu. 1948.

摘　　要

小山鐵夫・B. C. ストーン: ハワイ諸島のホタルイ属

ハワイ諸島のホタルイ属については今迄にまとまったものはなく，1888年刊行のヒレブランドのフロラには2種が記載されているに過ぎない．私達は今回主としてホノルルのビショップ博物館所蔵の標本をもとにしてハワイ諸島のホタルイ属全部をまとめた．ハワイには結局4種2変種を産し，このうち2種1変種は米国西岸から南米に分布し，2種は太平洋諸島に広く分布する．ホタルイの1変種 var. *Rockii* はハワイの山地に生ずるエンデミックであるが，その産地はカウアイ島の山地でアジア温帯要素の植物の見られる高地に限られる．これが日本の山地に生ずる近似種（ミヤマホタルイ）と外形上同様の習性を示すことは生態学的に興味深い．分類学上の新知見は学名およびそのノートによって表現した．（東京大学理学部植物学教室，ハワイ大学植物学教室）

Bot. Mag. Tokyo, **73**: 295–299 (July 25, 1960)

Studies on Seed Protein by Means of Turbidometric Titration, Especially in a Group of *Brassica rapa* L.

by Yoshihide MOMOTANI*

Received January 16, 1960

A prompt and simplified qualification of seed protein seems to be very important in the study of the following problems: how many protein fractions are present in a single seed, whether the individual fractions show some characteristic variation or not, and whether the composition of protein as a whole is fixed within a race.

In previous papers[1]), the author described the analysis of protein by means of titration curves obtained by using ammonium sulphate solution as a precipitant. Besides, a suitable method for the subfractionation of serum protein was also presented. This is named turbidometric titration. Recently, the turbidometric titration of synthetic polymers has been also reported by Morey *et al.*, Harris *et al.*, Dunn *et al.* and Melville[2]).

In the present paper, seeds from a group of *Brassica rapa* L. were investigated by this method in respect to globulins in each grain. The results showed that conspicuous difference was observed among several races as regards the nature of seed globulin. This seems, therefore, to provide a usefull tool for the study of affinity in plants.

Materials and Method

Seeds from some pure lines of *Brassica rapa* L. were used as material (cf. explanation of Fig. 5)[3]).

The solvent for protein composed of 1 per cent aqueous sodium chloride, and 25 per cent (v/v) of saturated ammonium sulphate (at 20°), and buffered with 0.25 M of K_2HPO_4–KH_2PO_4 to pH 7.0.

The ammonium sulphate solution used as a precipitant was 75 per cent (v/v) solution of saturated ammonium sulphate containing 0.25 M of K_2HPO_4–KH_2PO_4 buffer.

The principle of this method lies in an application of the turbidometric titration curves to the analysis of a small amount of protein solution by means of precipitation method, instead of differential salting out[4,5]).

The turbidometric apparatus consists of the followings: a glass cell having a capacity of 16 m*l*. and a light path of 30 mm. long, a microburette through which the precipitant is put into the glass cell, and the phototube of an electrophotometer to catch the light beam through the glass cell. The electrophotometer is equipped so as to read the difference of turbidity before and after each step of the titration experiment. A stirrer rotating at 100 r.p.m. is inserted into the glass cell, which is tightly fixed in order to avoid mechanical disturbance.

A solution containing 0.001 per cent of protein is taken into the glass cell, and the calculated volume of ammonium sulphate solution is added through the microburette. The mixture is thoroughly stirred for a few minutes, and the turbidity is measured at regular intervals. Following this first step of titration, the next calculat-

* Botanical Institute, Faculty of Science, Kyoto University, Kyoto, Japan.

ed volume of ammonium sulphate solution is added, and the turbidity is measured again. The same process is repeated, and the quantity of protein salted out in each step is carefully estimated during the entire course of titration experiment.

As a protein solution becomes diluted by the addition of aqueous ammonium sulphate, the turbidity measured at each step is adjusted on a fixed volume basis according to the Lambert-Beer equation within the range[1]), in which it holds good. The calibrated values of turbidity (T) are plotted against the percent concentration (v/v) of saturated solution of ammonium sulphate (C). The increment of turbidity per increment of salt concentration ($\Delta T/\Delta C$) is plotted against **C**, as shown in Fig. 1–1 and Fig. 1–2. The number of peaks on each curve in these figures corresponds to the number of component proteins.

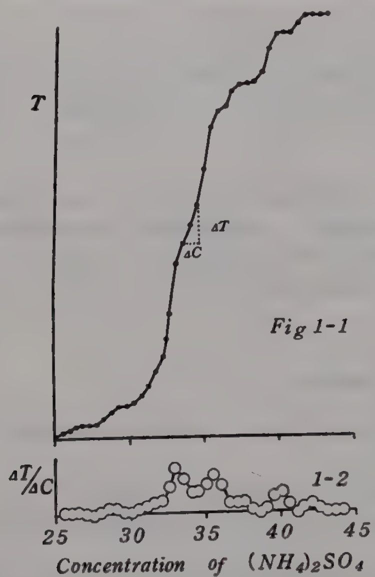


Fig. 1–1. Increase of turbidity (T) corresponding to an increase of concentration (C) represented by per cent (v/v) of saturated ammonium sulphate.

Fig. 1–2. Increment of turbidity at various concentrations of ammonium sulphate. ΔT shows the difference between the values of turbidity before and after each step of titration. ΔC is the variance of the concentration of ammonium sulphate.

Results and Discussion

By means of the procedure stated above, the nature of globulin in each seed was carefully compared.

The results have shown that the similar $\Delta T/\Delta C$-curves can be obtained from the seeds within the same pure line, as depicted in Figs. 2, 3, and 4. In fact, each globulin is composed of several components.

From the comparison of the curves obtained from several pure lines, the majority of globulin components are very similar to each other within the lines, whereas some are apparently characteristic for each line (Fig. 5).

Although some variation may be observed in relative amount of globulin components, the presence or absence of specific components is found to be definitive within the same line (Figs. 2, 3 and 4).

Various seeds in the market were found, in general, to be similar with each other as regards their globulin components, while some of them gave quite different $\Delta T/\Delta C$-curve (Fig. 6–1). A representative $\Delta T/\Delta C$-curve is shown in Fig. 6–2 as the standard, and the curve of a globulin fraction obtained from 50 seeds is shown in

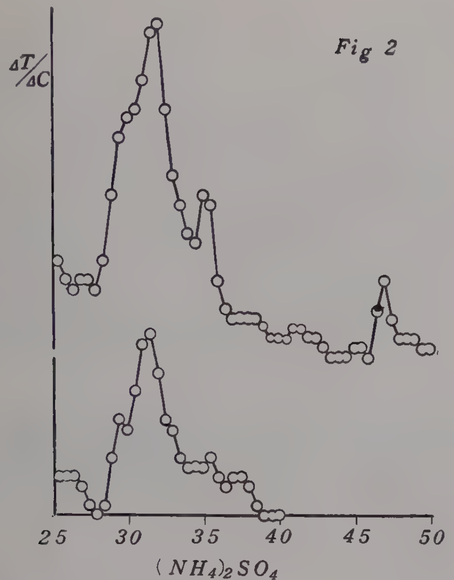
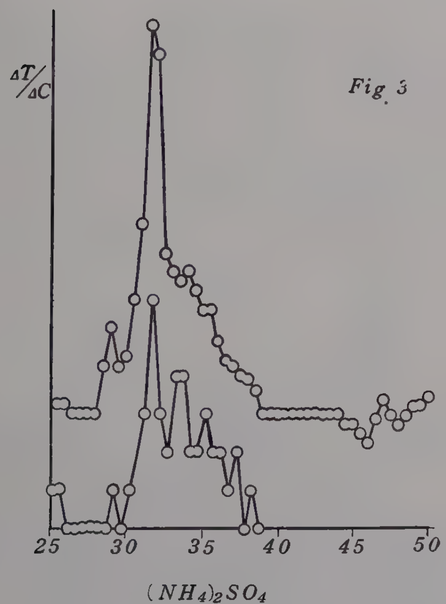


Fig. 6–3 (the solution is diluted twenty times as much with the same solvent). The curves in the last two figures resemble each other.

The seeds of hybrid line showed apparently combination or segregation of some globulin components, exact data for which will be provided by further experiment. The same specificity has not been found in the case of electrophoretic examinations. It may be of importance to carry out further study on vegetable protein from immunogenetic point of view. At any rate, it is shown from the present experiment that the seed globulin of *Brassica rapa* group consists of a number of components, and the majority of them appear to be genetically fixed, and that the globulin is specific for each of the breeding lines (Fig. 5).



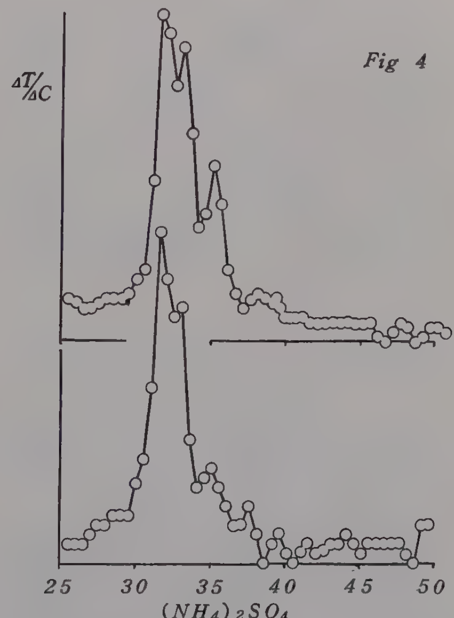


Fig. 2, Fig. 3 and Fig. 4. Reproducibility of turbidometric analysis on a single seed from the same pure line. Fig. 2: Hiroshimana (4X), Fig. 3: Hiroshimana (2X) and Fig. 4. Tyôsen-hakusai.

Here, it should be noted that the turbidity is not always proportional to the amount of protein salted out; the value of ΔT is sometimes negative against positive value of ΔC. Such a disturbance can be eliminated to some extent by decreasing the concentration of protein, and almost completely by the addition of some chlorine ion[1]).

It is not yet sufficiently established whether or not the turbidity at each titration step is brought into equilibrium in the salting out reaction. The rate of turbidity

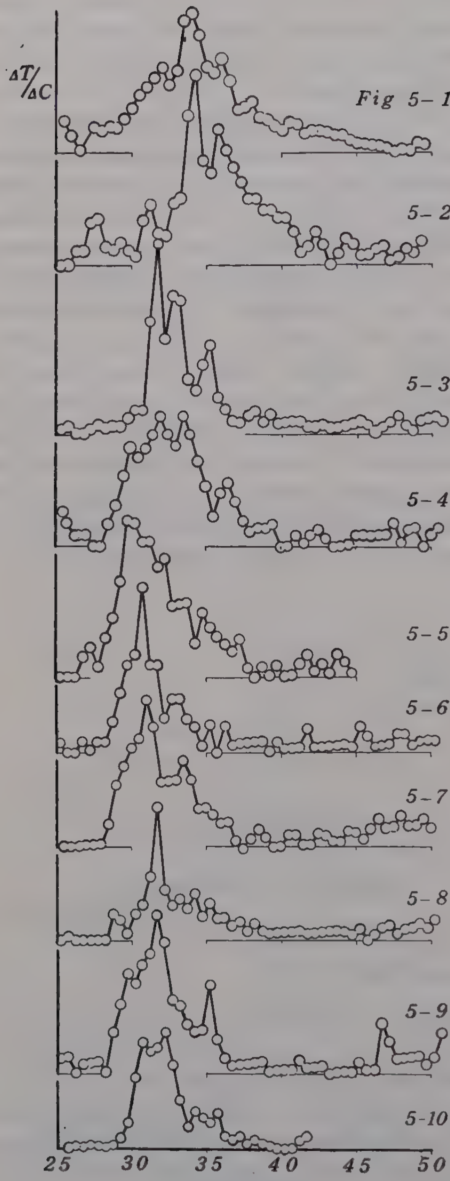


Fig. 5. Comparison of globulin components found in the pure lines. 5-1: Tennôji-kabura, a pure line in *Brassica rapa*, var. *glabra* Kitamura. 5-2: Shôgoin-kabura in var. *glabra* K. 5-3: Honkô-akadaikon in var. *japonica* K. 5-4: Tyûsei-mibuna in var. *laciniifolia* K. 5-5: Nikanmetaisai in var. *chinensis*, subvar. *longipetiolata* K. 5-6: Taisai in var. *chinensis* K. 5-7: Taisai × Hiroshimana. 5-8: Hiroshimana in var. *amplexicaulis*, subvar. *Hiroshimana* K. 5-9: Hiroshimana (4×). 5-10: Tyôsen-hakusai in var. *amplexicaulis* Tanaka *et* Ono.

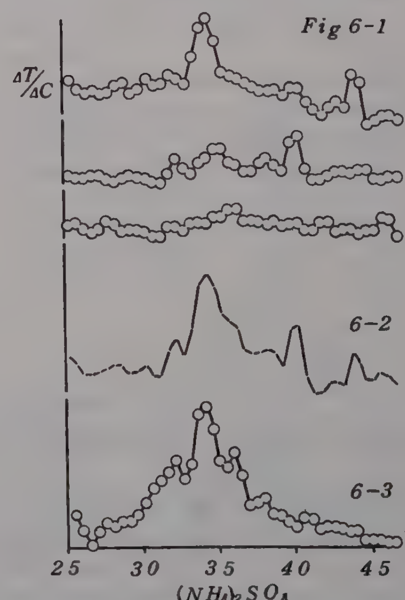


Fig. 6. Variation of seed globulins.

caused by ammonium sulphate is apt to change within a minute. In practice, the increment of turbidity may be usually negligible and or even nullified after standing for 5 minutes. The turbidity reaches a steady state after 25 minutes. Therefore, titration was made at regular intervals of 5 minutes. The titration made at intervals of 20 minutes was found to be the same as above. In cases of more than 0.1 per cent concentration of protein, the turbidity continued to increase even after standing for 20 minutes.

Summary

Salting out behaviour of seed globulins in a group of *Brassica rapa* L. was studied turbidometrically using ammonium sulphate as precipitant. By this means, the amount of seed globulin salted out could be easily measured stepwise during the whole course of experiment. The increment of turbidity per increment of salt concentration ($\Delta T/\Delta C$) was plotted against various concentrations of ammonium sulphate (C), as shown in the figures illustrated above. By this method the seeds were investigated in respect to globulins in each grain. From the comparative study of these curves, it was concluded that in every pure line of *Brassica rapa* group the seed globulin is composed of several components, most of which are closely similar to those obtained from other lines, and that some characteristic components are also present in each line.

Cordial thanks are due to Prof. S. Kitamura for his guidance and helpful suggestions throughout this work. Thanks are also due to Prof. I. Nishiyama for the supply of seeds from some pure lines.

References

1) Momotani, Y., and Sogami, M., Bot. Mag. Tokyo, **67**: 178 (1954), and Sogami, M., Momotani, Y., and Inoue, Y., Jour. Biochem., **44**: 137 (1957). 2) Morey, D. R., and Tamblyn, J. W., Jour. Appl. Phys., **16**: 419 (1945), ——, Taylor, E.W., and Waugh, G.P., Jour. Colloid Sci., **6**: 470 (1951), Harris, U., and Miller, R. G. J., Jour. Polymer Sci., **7**: 377 (1951), Dunn, A. S., Stead, B. D., and Melville, H. W., Trans. Farad. Soc., **50**: 279 (1954), and Melville, H. W., and Stead, B. D., Jour. Polymer Sci., **16**: 505 (1955). 3) Kitamura, S., Mem. Col. Sci. Univ. Kyoto ser. B **XIX**: n. 3 art. 16 (1950). 4) Derrien, Y., Biochim. Biophys. Acta 8: 631 (1952), Steyn-Parve, E. P., and Van Den Hout, A. J., Biochim. Biophys. Acta **10**: 320 (1953), and Amirkhanova, S. N., and Amirkhanova, A. Kh., Biokhimiya **19**: 19 (1954). 5) Ventura, M. M., and Hollanda Lima, I., ϕton 8 (2): 137 (1957).

摘　　要

桃 谷 好 英: 種子蛋白の濁度滴定(特にカブラ近縁植物の種子について)

*Brassica*の種子はかなり小さいが，一粒ごとに種子蛋白の組成をしらべることができれば，蛋白質の種属特異性や，各蛋白成分の遺伝や環境などによる変化について知る上に役立つと考えてこの研究を行なった.

種子蛋白の溶液(蛋白量 0.001%, NaCl 1% を含み，硫酸アンモニウム 25%飽和)に硫酸アンモニウム溶液(75%飽和)を滴下しながら塩析された蛋白質の量を(Turbidometry によって)記録する．硫酸アンモニウムの種々な濃度(C)で塩析された蛋白質の量の変化($\Delta T/\Delta C$)は，一見，電気泳動図に似た曲線になるので，この図形から *Brassica rapa* group の各純系の種子から抽出されたグロブリン中の構成分画を比較した(電気泳動では図のような多くのピークは得られなかった)．図の曲線には多くのピークが見られるが，各ピークはグロブリンの中の一成分に対応すると見てよい[1,2].

種子グロブリンは各純系ごとにほぼ一定の構成をもち，品種内では互いによく似ているが，品種間では差が認められた．なお，この濁度滴定法によって種子以外の蛋白質試料(例えば，アルブミン，組織蛋白あるいは血清など[1]))の分画もしらべることができた．(京都大学理学部植物学教室)

Bot. Mag. Tokyo, **73**: 300–309 (July 25, 1960)

Intraspecific Competition in Artificial Sunflower Communities*

by Sumio KUROIWA**

Received January 23, 1960

In the study of intraspecific competition in an *Abies* forest on Mt. Shimagare[1–3]), causal analyses have been made regarding stand density, frequency distribution of tree size, and difference in productivity among tree size classes. The author, however, has experienced several difficulties in a logical comprehension of these problems because of variability in the physiological characteristics and uncertainty of habitat conditions. A simplified plant community which can be artificially controlled is an absolute necessity for fundamental analysis of the problem.

By means of artificial plant communities, interspecific competition has been clarified by Iwaki[4]) on the basis of dry matter production, and intraspecific competition was studied by Clements *et al.*[5]) and recently by Kira *et al.*[6,7]). The experimentation based on dry matter production should also be achieved on intraspecific competition (cf. Boysen Jensen[8]) and Satoo *et al.*[9,10])).

In an artificial sunflower community the author has studied intraspecific competition on the same line as in the previous studies[1–3]), clarifying that the cardinal factor in intraspecific competition, a universal phenomenon in social life of plants, is the productivity difference among the plants belonging to different size classes, which is caused by the vertical light distribution in the community.

Methods and Material

Field experiments were carried out in Tokyo, in the summer seasons of 1957 and 1958. Before sowing, manure and chemical fertilizer were applied to experimental fields enough to minimize the competition for mineral nutrition. The plant used here was *Helianthus annuus* L. (variety; "Large-Russian"). The seeds were sown in a regular square disposition of four kinds of spacing[4,11,12]), 5, 7, 10, 20 cm. in both directions, or 400, 200, 100 and 25 plants/sq. m. The plot areas were 3×2, 5×3, 5×4 and 5×5 sq. m., respectively. The sowing dates were at the 5 cm. plot Sept. 2, 1957, at the 10 cm. plot July 5, 1957 and at the 7 and 20 cm. plots July 1, 1958. The communities in all plots were set up with a normal frequency curve of seed weight (m=35 mg., σ=13.9).

About 50 plants included within a rectangular frame laid down were measured in height and weight. Out of them a few plants were taken out from each plant class as representatives, and cut into several strata, then divided into leaves, stems, petioles and roots, measured in their fresh and dry weights after the stratifying clip method[13]) (cf. also a previous paper[1]). On the other hand, as done by Kira *et al*[7].), the plants were estimated in dry weight without injuring them by stem volume index (=square of stem basal diameter × stem height), using a curvilinear relation determined beforehand between those two measures (Fig. 1). Photosynthesis of leaves and respiration of each organ were measured by the partially modified Boysen Jensen method[2,8,14]) (at 0.03 CO_2 vol. percent). Electric photometers (Toshiba No. 5) were

---

* Supported by the Grant in Aid for Scientific Research from the Ministry of Education.
** Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.

used for the measurement of illumination in photosynthesis experiments as well as in determination of vertical light distribution in the sunflower stand.

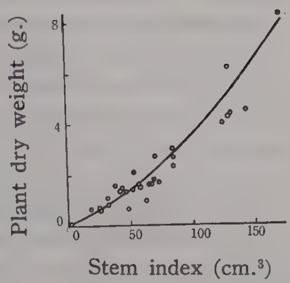


Fig. 1. Relation between dry weight of total aerial part and stem volume index (square of basal stem diameter × stem height) in constituent plants of a sunflower stand (100 plants/sq. m.). 34 days after sowing.

Stability of ranks of constituent plants in their height and weight

The *Abies* trees defeated in intraspecific competition were found not only in the smallest class but also in the larger classes[1]). There, the rank in tree size of the constituent trees should be variable with the development of the stand. Such a mode of self-thinning and an unstability of the rank in tree size might be due to random distribution of the *Abies* trees, because in the narrower spacing the mutual shading of the canopies causes severer competition for environmental factors affecting matter production (Iwaki[4]), Hogetsu *et al.*[12])). As for self-thinning in the artificial sunflower community with regular disposition, it is important to clarify whether the rank of plant in size is stable during the development of the community.

In the 10 cm. plot (100 plants/sq. m.), about 50 plants were measured in height, and estimated in weight by the foregoing method at three growth stages, 22, 34 and 46 days after sowing. The values of correlation coefficient obtained by Spearman's rank-difference method[15]) were 0.82 in plant height and 0.79 in plant dry weight between the early and the middle stage, and 0.89 and 0.95 between the middle and the late stage, respectively. These high correlations indicate that the ranks, which were determined in the initial growth stage, of the sunflower plants in their height and weight were invariable in the regular disposition throughout the stand development. Moreover, the larger the seed in dry weight, the larger was the seedling grown from it (Tab. 1). Accordingly, it is conclusive that in an even-aged stand with regular disposition the constituent plants were destined to maintain the same rank in their height and weight as initially determined regarding seed dry weight.

Table 1. Relation between seed and seedling of 10 days old, in dry weight (mg.). Average of 20 individuals. Figures in parentheses indicate the relative values.

Seed weight	Seedling weight	Ratio
68 (4.0)	101 (3.7)	1.48
58 (3.4)	88 (3.3)	1.52
43 (2.5)	59 (2.2)	1.37
17 (1.0)	27 (1.0)	1.59

Within the constituent plants with stable ranks in the measures, the self-thinning should occur only in the plants of the smallest class, because such plants are generally placed at the most disadvantageous position of the stand in light factor and consequently in dry matter production, as shown in previous papers[1, 3]). Actually, several suppressed sunflower plants belonging to the smallest class of the densest stand were defeated in the latest stage (see the latest chapter).

Frequency curves of plant measures dependent on plant growth rate

The above-mentioned stability of rank in measures, e.g. height and dry weight, implies that the difference between the measures at two growth stages of the plants

Table 2. Plant dry weight W, plant height H, leaf area $\bar{F}$, daily increments (ΔW and ΔH), relative growth rate (RGR = daily increment/plant measure), and net assimilation rate (NAR = daily increment/leaf area) of sunflower plants, 30 and 40 days after sowing. The plants were classified into three classes, dominant D, intermediate I, and suppressed S, at the initial growth stage (16 days after sowing).

		Class	W (g)	H (cm)	$\bar{F}$ (cm²)	ΔW (mg/day)	ΔH (mm/day)	W-RGR (mg/day/g)	H-RGR (mm/day/cm)	NAR (mg/day/cm²)
200-plant stand	30 days	D	2.5	65	453	320	43	130	.66	.71
		I	.75	44	180	40	25	54	.57	.22
		S	.25	26	92	6.2	12	25	.45	.07
	40 days	D	6.55	114	790	520	55	80	.48	.66
		I	1.0	70	192	23	25	23	.36	.12
		S	.28	37	62	0	9.6	0	.26	0
25-plant stand	30 days	D	3.4	34	710	510	27	150	.80	.72
		I	2.4	28	590	310	22	130	.80	.52
		S	1.2	21	360	110	16	95	.75	.31
	40 days	D	12.4	88	1640	1340	115	110	1.3	.82
		I	6.6	68	1065	495	82	75	1.2	.46
		S	1.8	42	410	49	30	27	.7	.12

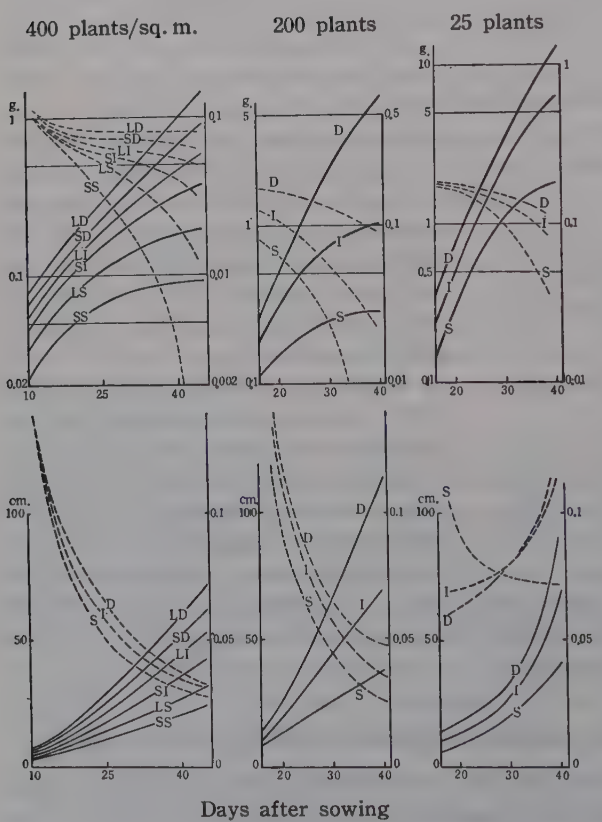


Fig. 2. Growth of sunflower plants of three even-aged stands, 400, 200 and 25 plants/sq.m. Left ordinates and solid lines, plant dry weight (upper) or height (lower). Right ordinates and broken lines, daily relative growth rate of each measure. *D*, *I*, *S*, dominant, intermediate and suppressed, respectively. In the 400-plants stand each class was subdivided into large (*L*) and small (*S*) plant classes.

belonging to the same class corresponds to their growth in the measure concerned. Fig. 2 shows time course of the two measures and of their relative growth rate (cf. Tab. 2). The constituent plants were classified on a dry weight basis at the initial growth stage (16 days after sowing) with normal frequency curve. As for plant dry weight, the dominant, specially of the sparser stand, kept an almost constant relative growth rate, but the suppressed rapidly decreased the growth rate in the course of time, especially in the denser stand. In the relative height growth rate, no such marked difference as observed about dry weight was found between plant classes of every stand over the whole growth stage. In a mathematical analysis of intra-specific competition, it was assumed by Kira *et al.*[6] that every plant class kept a relative weight growth rate constant throughout the whole growth stage, small plants relatively holding a lower rate. However, the author obtained the result that only the dominant sunflower plants could keep a constant growth rate, and those in smaller classes, especially the suppressed, rapidly decreased the weight growth rate with depression of matter production.

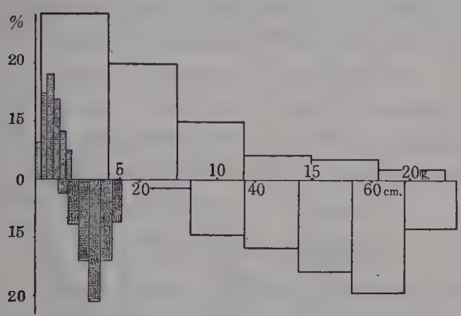


Fig. 3. Frequency histograms of fresh weight of total aerial part (upper) and plant height (lower), in an early growth stage (17 days after sowing, dotted polygons) and in a late stage (45 days after sowing, blank polygons) of an even-aged sunflower stand (400 plants/sq.m.).

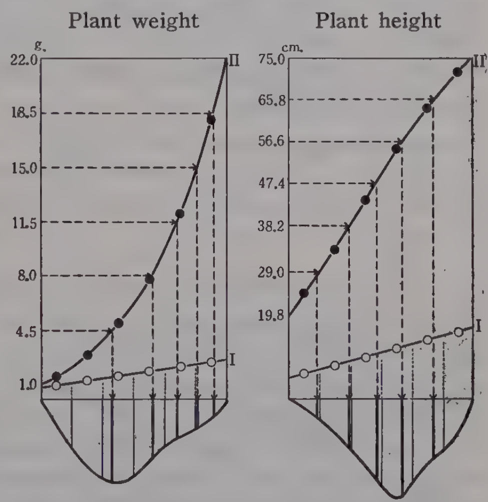


Fig. 4. Change of frequency distributions of plant measures (fresh weight of aerial part, and height) consequent on growth difference among sunflower plant classes in the 400-plant stand (cf. Fig. 3). Open circles on line I and vertical fine solid lines indicate respectively values of plant measures and classification of frequency curve in an early growth stage (17 days after sowing). Solid circles on curve II and vertical thick solid lines indicate those in a late growth stage (45 days after sowing). The measures of average plants of classes divided by the first classification have changed from line I to curve II after 28 days. The new classification with the same intervals has to be made for the new amplitude of the measure being shown by the horizontal arrows. The projection of this classification upon the first frequency curve gives rise to the new frequency distribution. The area between thick solid lines in the first frequency curve indicates the real frequency in the new classification.

The frequency curves shown in Fig. 3 were constructed with height and fresh weight of aerial part which were actually measured in the 400-plant stand. The frequency curve of fresh weight transformed from the N-type to the L-type (these were designated by Kira *et al.*[6]) in the early period of growth, skewing more strikingly with stand development. The plant height, however, maintained the normal frequency curve throughout the period of development. These facts, which well accord with the results obtained by Kira *et al.*[6,7], will be elucidated with increas-

ing difference in weight growth rate and with invariable equality in height growth rate among plant classes. Provided the growth curve is known in each plant class, the change of frequency curve can easily be estimated by the drawing method illustrated in Fig. 4. Also in the 25– and 200–plant stands this drawing method was adopted with the plant growth in Fig. 2 to confirm the changes of frequency distributions, and the results were more or less the same as obtained in the 400–plant stand mentioned above. Moreover, the difference consequent on plant density appeared in the skewing of the plant weight frequency curves, but not in the plant height curves that remained in the normal type throughout the experiments.

Role of light factor in intraspecific competition

Boysen Jensen[8]) already suggested a great importance of light factor in competition in the plant community. Recently Iwaki[4]) and the author[1, 3]) could clearly confirm that the lower productivity of the suppressed plants, which would often decide their fate, was mainly caused by their light condition deteriorated with shading by the canopies of dominant plants. In the sunflower stand concerned, therefore, the intraspecific competition should be studied with special consideration for light factor related to matter production.

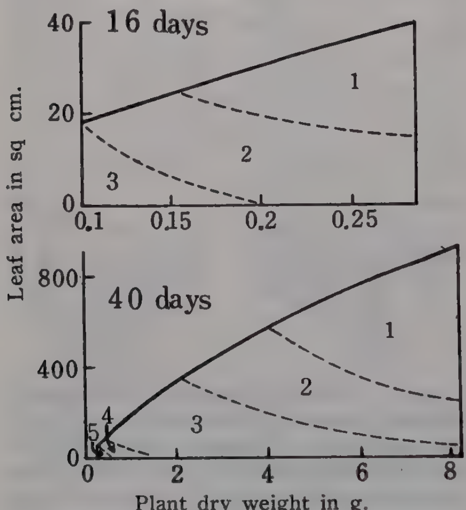


Fig. 5. Relation between the total dry weight and the leaf area of a sunflower plant. The 200-plant stand. After 16 days from sowing (upper): leaf stratum 1: 15–10 cm. high, 90% of relative light intensity, 2: 10–5 cm., 70%. 3: 5–0 cm., 55%. After 40 days from sowing (lower): leaf stratum 1: 120–100 cm. high, 85% of relative light intensity. 2: 100–80 cm., 60%. 3: 80–60 cm., 32%. 4: 60–40cm., 15%. 5: 40–20 cm., 5%.

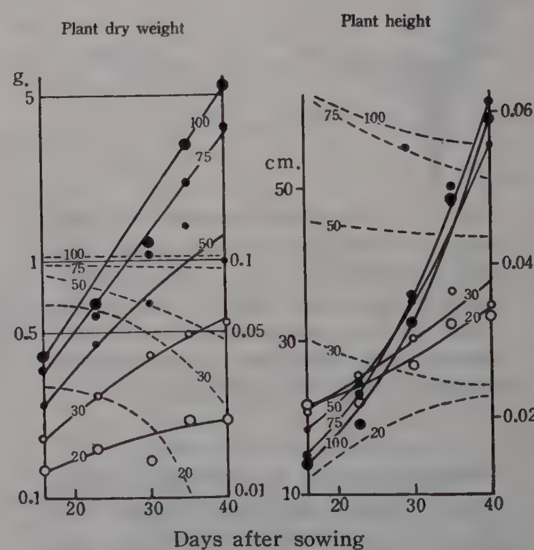


Fig. 6. Growth (solid lines—left ordinates) and the relative growth rates (broken lines—right ordinates) of sunflower plant in relative light intensities of 20, 30, 50, 75 and 100 per cent. Mean values for 10 plants.

The vertical distribution of leaves within the stand was clarified in plant belonging to each size class. Fig. 5 shows the relation between the total dry weight and the whole leaf area of plant, and the position of leaves of each plant with varying dry weight. At all growth stages, the heavier the plant in dry weight, the larger was it in whole leaf area, distributing the larger portion of the latter to higher strata of the stand. The larger the plant in weight, the higher was the intensity of light

received by its foliage. This trend became conspicuous with stand development. Such a difference in light factor should be found not only between plant weight classes but also between plant height classes, because taller plants are generally heavier in weight.

In connection with shade tolerance, many workers[5, 16–33] have studied experimentally the shading effect on plant growth, and observed, except for a few shade tolerant species, severe growth depression by shading, notably in sun plants. Such characteristics of shade tolerance may also have a close relation with the bearing of the plants in intraspecific competition in a closed plant stand. The sunflower plants were examined in dry weight growth as well as in height growth, and in each relative growth rate, under a series of screens (Fig. 6). The seeds were sown on July 1, 1958, with spatial interval sufficient to avoid the mutual shading with neighboring plants. The daily increment and the relative growth rate in weight decreased markedly with shading, but those in height were rather enhanced with screening at the early stage. At the late stage, however, the plants grown under 50, 75 and 100 per cent illuminations reached almost the same height, but those under 30 and 20 per cent illuminations were remarkably shorter. The leaf area development indicated a similar trend to the weight growth.

These bearings of the sunflower plants under varying illumination, together with the vertical distribution of leaf and light in the stand, seem well to elucidate the difference in the weight and the height growth between the plant classes of the stand. The rapid decrease in weight growth rate of the plants grown in a 20 per cent illumination suggests the fate of the suppressed plants in increasing shade under the dominant's canopy.

Analysis of different productivity between plant classes

Plant growth progresses through the interaction between physiological functions and environmental factors. Accordingly, ecological problems as to plant growth should be analysed from these two aspects and the obtained results should again be synthesized on the basis of matter production[8]. Such integrating procedure has brought about a better understanding of density effect (Iwaki[11]), Hogetsu *et al.*[12]), interspecific competition (Iwaki[11]) and intraspecific competition in an *Abies* forest (Kuroiwa[1–3]). This was applied to the present study according to the procedure similar to that in a previous one[3]).

The data obtained for matter production of the sunflower plants are summarized in Tab. 3 and Figs. 7 (respiration) and 8 (photosynthesis). Some remarks should be given here. In the leaf area ratio designated by Blackman[26–29]) (the ratio of the whole leaf area $\bar{F}$ to the plant dry weight W, one of the factors deciding the relative weight growth rate), the suppressed is higher than the dominant because of larger value of leaf weight ratio (Watson[34]), $\bar{F}$/ leaf dry weight F). There were not observed any other marked differences in the concerned characteristics. In conclusion, the principal cause for the observed marked depression in relative growth rate of suppressed plants seems to be the low actual photosynthesis resulting from the difference of illumination received between plant classes (cf. p. 168 in a previous paper[3])).

In order to prove the above-mentioned conclusion exactly, resynthesis of weight growth was made in each plant class, with combining physiological data and environmental factors[3,4,11,12]). Daily gross photosynthesis per unit leaf area P_a at a certain height was calculated as $P_a = KbI_{M}e^{-KF}/(1+KaI_{M}e^{-KF})$, where a and b are constants, I_M is a daily maximum illumination, K is the extinction coefficient characteristic of a plant community (K=1 in the sunflower stand), and F is the leaf area index calculated

from the top of foliage to the height concerned (cf. Monsi and Saeki[13]), Davidson and Philip[36]), and Saeki[33]). The calculated value is represented against F in the Fig. 9 with a solid line (I_M=100 kilolux, daily mean temp.=25°). The figure also illustrates the leaf area distribution in each stratum and in each plant class of the

Table 3. Leaf area ratio ($\bar{F}/W$), leaf weight/plant weight ratio (F/W), and leaf weight ratio ($\bar{F}/F$), in the representative sunflower plants shown in Fig. 2 and Tab. 2. Signs, D, I, S, W, $\bar{F}$, are the same as in Tab. 2. F indicates g. leaf dry weight per plant. $\bar{F}/W=F/W\times\bar{F}/F$.

Days after sowing		200-plant stand 16	200-plant stand 30	200-plant stand 40	25-plant Stand 16	25-plant Stand 30	25-plant Stand 40
$\bar{F}/W$	D	146	181	121	138	208	132
	I	150	239	190	147	245	161
	S	182	374	222	159	298	226
F/W	D	0.49	0.42	0.36	0.58	0.55	0.46
	I	0.49	0.41	0.32	0.56	0.54	0.44
	S	0.49	0.41	0.29	0.50	0.48	0.44
$\bar{F}/F$	D	297	436	336	239	380	287
	I	308	580	592	262	450	365
	S	372	912	766	314	620	514

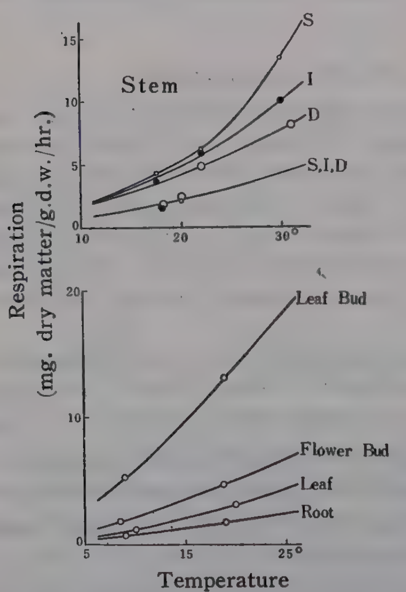


Fig. 7. Temperature-respiration curves in matured sunflower plants of the 200-plant stand. Above: upper three curves show the difference in upper part of stem among plant classes (D, dominant; I, intermediate; S, suppressed), and the lowest curve indicates the equivalence in its lower part among them. Below: respiration in various organs of a plant belonging to the intermediate class.

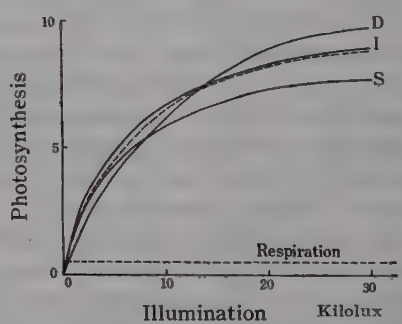


Fig. 8. Light-photosynthesis curves in mature leaves of matured sunflower plants. Each curve was drawn with ca. 10 measurements obtained in a few plants belonging to each plant class in the 200-plant stand. Signs mean the same as in Fig. 7. The photosynthesis curve used for the calculation of daily production is given by the broken line. Ordinate: mg. dry matter/100 sq. cm. leaf area/hr. at 20°.

sunflower stand, with combining the plant number of each weight class in Tab. 4 and the vertical leaf distribution in Fig. 5.

The total daily photosynthate produced by all the leaves in each stratum was shared to each plant class, in proportion to the area of the leaves concerned. The shares of photosynthate of all the strata were summed up in each plant class, which brings about the daily total amount of dry matter production in each plant class. This amount was divided by the plant number concerned, and thus the mean daily gross production per individual of each class was obtained.

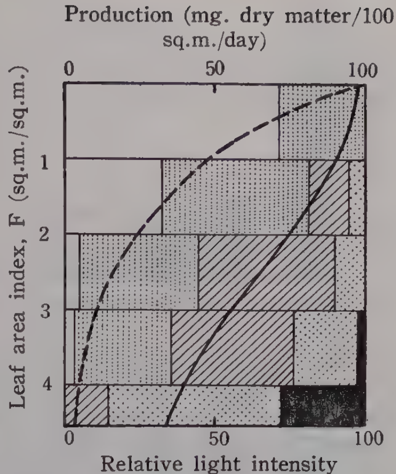


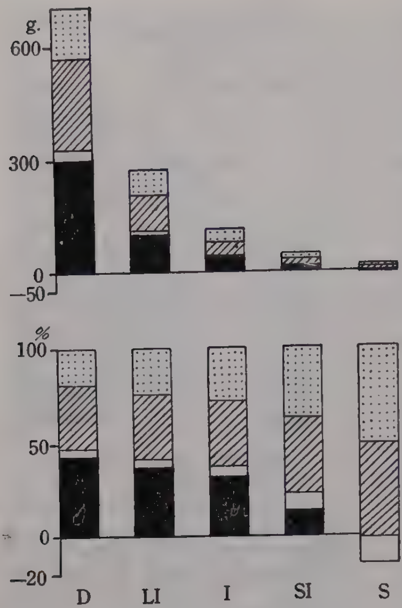
Fig. 9. Relation of leaf area index (F) to relative light intensity (broken line) and to daily matter productivity (solid line) in the 200-plant stand, 40 days after sowing. The figure also indicates the distribution of leaf area among plant classes shown in Tab. 4 (D: blank polygon, LI: densely dotted, I: hatched, SI: sparsely dotted, S: black.)

Daily respiratory loss of matter in leaves, stems and roots was calculated with combining the dry weight of each organ with its respiration rate, practically of the intermediate plant in Fig. 7, at daily mean air temperature. The difference between the daily gross assimilation and daily respiratory loss corresponds to the daily increment of plant in dry weight. The calculated values for each plant class of the 200-plant stand at the latest stage of the experiment (40 days after sowing—Aug. 10, 1958—fair; max. illumination 100 kilolux; mean temp. 25°) were summarized in Fig. 10 and Tab. 4.

Table 4. Daily gross and net production in sunflower plants. The 200-plant stand, on August 10, 1958 (40 days after sowing—fine, daily max. illumination 100 kilolux, daily mean temp. 25°). Number of plants in each class was determined at the initial growth stage (16 days after sowing): D (dominant, 16 plants/sq. m.), LI (larger intermediate, 32), I (intermediate, 58), SI (smaller intermediate, 64), and S (suppressed, 30).

	Class	Dry weight	Gross production	Respiration				Net production
				Leaves	Stems*	Roots	Sum	
Individual (mg./plant)	D	6550	700	132.6	239.0	30.0	401.6	298.4
	LD	2520	271	65.7	92.2	12.6	170.5	100.5
	I	1030	113	32.3	38.9	7.2	78.4	34.6
	SI	550	47.5	17.5	18.7	4.8	41.0	6.5
	S	280	21.4	10.7	10.8	3.0	24.6	− 3.2
Stand (g./sq. m.)	D	105.0	11.19	2.12	3.82	0.48	6.42	4.77
	LD	80.5	8.66	2.10	2.95	0.40	5.45	3.21
	I	59.7	6.57	1.88	2.26	0.42	4.56	2.01
	SI	35.2	3.04	1.12	1.20	0.31	2.63	0.41
	S	8.4	0.64	0.32	0.33	0.09	0.74	−0.10
	Sum	289	30.10	7.54	10.56	1.70	19.80	10.30

* Petioles are included to the stems.



The dominant class is superior in the gross and the net production to any other classes. The 16 dominant plants out of 200 plants in a square meter contributed 46 per cent to the total daily gross production of 30 g., and 46 per cent to the total daily net production of 10.3 g. On the contrary, the 30 plants of the suppressed class occupied only 2 per cent in gross production and performed rather negative net production because of larger respiration amount compared with the photosynthesis.

Fig. 10. Daily gross production (whole polygon) and net production (black portion) of representative sunflower plants in g. dry matter/plant. The 200-plant stand, 40 days after sowing (cf. Tab. 4). Dotted, hatched and blank portions indicate respectively the respiratory losses of dry matter in leaves, stem and petioles, and roots. Above: absolute values. Below: relative ones.

In each plant class, thus calculated values in matter production considerably well accord with the daily weight increment and relative weight growth rate which were obtained by the actual measurement of plant growth (compare Tab. 2 with Tab. 4). Also in the 400-plant stand, such accordance was found in the earlier growth stage as well as in the later. In conclusion, the difference in relative weight growth rate between plant classes can be logically elucidated by taking microenvironmentally the difference in light factor between them into consideration. This proves very clearly the importance of light factor in the growth competition.

Summary

1) Intraspecific competition was studied in artificial sunflower stands with four kinds of densities. The stands were set up with normal frequency curves of the seed weight.

2) The rank of the constituent plants in plant dry weight and height was destined to keep the same rank as in the initial weights of sown seeds. Self-thinning occurred only in the smallest class with the worst light condition for matter production.

3) Skewing of frequency curve of plant weight was induced by the difference of weight growth rate among the plant classes, while maintenance of normal frequency curve in plant height was resulted from the equality of height growth rate among them.

4) The differences among the plant classes were discussed of photosynthetic and respiratory activities, and of other characteristics concerned with matter production. However, they were not so conspicuous as to give rise to the difference among the classes. The most striking factors concerned were the vertical distribution of light and of leaves in the stands.

5) The importance of light factor in intraspecific competition was proved by the shading experiments, and furthermore by calculation of difference in daily matter production among plant classes with combining the photosynthetic and respiratory

rates and light (and temperature) factors determined experimentally.

The author is much obliged to Prof. M. Monsi for his judicious advice and valuable guidance, and to Assistant Prof. S. Yokogi for his making facilities available for carrying out this work.

References

1) Kuroiwa, S., Bot. Mag., Tokyo **72**: 413 (1959). 2) ——, ibid. **73**: 133 (1960). 3) ibid. **73**: 165 (1960). 4) Iwaki, H., Jap. Jour. Bot. **17**: 120 (1959). 5) Clements, F. E., Weaver, J. E., and, Hanson, H. C., Plant Competition, Car. Inst. Wash. (1929). 6) Koyama, H., and Kira, T., Jour. Inst. Polytech., Osaka City Univ. **7**: 73 (1956). 7) Yoda, K., Kira, T., and H. Kazuo, ibid. **8**: 161 (1957). 8) Boysen Jensen, P., Die Stoffproduktion der Pflanzen, Jena (1932). 9) Satoo, T., Nakamura, K., and Senda, M., Bull. Tokyo Univ. Forest. No. 48, 65 (1955). 10) ——, Kunugi, R., and Kumekawa, A., ibid., No. 52, 33 (1956). 11) Iwaki, H., Jap. Jour. Bot. **16**: 210 (1958). 12) Hogetsu, K., Oshima, Y., Midorikawa, B., Tezuka, Y., Sakamoto, M., Mototani, I., and Kimura, M., Jap. Jour. Bot. **17**: 278 (1960). 13) Monsi, M., and Saeki, T., ibid. **14**: 22 (1953). 14) Saeki, T., and Nomoto, N., Bot. Mag. Tokyo **71**: 235 (1958). 15) Kendal, M. G., Advanced theory of Statistics (I), London (1948). 16) Lubimenko, W., Ann Sci. Nat. Bot. **9** (7): 321 (1908). 17) Combes, R., ibid. **9** (11): 75 (1910). 18) Garner, W. W., and Allard, H. A., Jour. Agr. Res. **18**: 580 (1920). 19) Gregory, F. G., Ann. Bot., **35**: 93 (1921). 20) Maximov, N. A., and Lebedincev, E. L., Ber. Dtsch. bot. Ges. **41**: 292 (1923). 21) Pop, H. W., Amer. Jour. Bot. **13**: 706 (1926). 22) ——, Bot. Gaz. **82**: 306 (1929). 23) Shirley, H. L., Amer. Jour. Bot. **16**: 354 (1929). 24) Blackman, G. E., and Templeman, W. G., Ann. Bot. N. S. **4**: 553 (1940). 25) Milthorpe, F. L., ibid. **9**: 31 (1945). 26) Blackman, G. E., and Rutter, A. J., ibid. **12**: 1 (1948). 27) ——, and ——, ibid. **14**: 487 (1950). 28) ——, and Wilson, G. L., ibid. **15**: 63 (1951). 29) ——, and ——, ibid. **15**: 373 (1951). 30) Mitchell, K. J., Physiol. Plantarum **6**: 21 (1953). 31) Bormann, F. H., The Physiology of Forest Trees, P. 197, N.Y. (1957). 32) Nomoto, N., and Iwaki, H., Biol. Sci. (Japanese) **9**: 34 (1957). 33) Kamel, M. S., Meded. Landbouwhogeschool, Wageningen **59**: 1 (1959). 34) Watson, D. J., Ann. Bot. N. S. **22**: 37 (1958). 35) Davidson, J. L., and Philip, J. R., Climatology and Microclimatology (P. 181), UNESCO (1958). 36) Saeki, T., Bot. Mag. Tokyo, **73**: 55 (1960).

摘　要

黒 岩 澄 雄: ヒマワリ人工群落における種内競争の解析

種内競争の解析[1-3]を縞枯山の *Abies* 針葉樹林で行なったがこの解析をより充分にするため，種子重につき正規分布をもったヒマワリ種子の正方形播きで4密度区 (400, 200, 100, 25本/m²) を作って種内競争を研究した.

個体重や草丈の順位は播かれた種子の重さの順位のままであることを Spearman の順位差法[15]による計算で得た高い相関度から推定し，高密度区の生育後期には小個体階級のみにおいて枯死体を観察した. *Abies* 森林での全階級にわたる枯死体の出現は，密度効果の解析結果[11, 12]から群落構成個体の間隔の不規則性によると推論した. 生育初期に個体重について階級分けされた各階級の平均個体の重さや草丈についての生長曲線を追跡し，重量生長率では高密度区ほど，また生育後期ほど大個体が小個体より大きく，草丈生長率では大差なかった. 重量度数分布はN型からL型[6]へと移行し，それは生育後期ほどまた高密度区ほど顕著であったが，草丈度数分布はほぼN型を維持していた. 他方，このような度数分布の時間変化を各階級の平均個体の重量生長を用いて，簡単な作図法で図示し，度数分布の変化は階級間での生長率の差によって引起されることを証明した. 同化能や呼吸能それに同化器官と非同化器官との量的関係についても階級間で大差なかったが，大個体ほど葉層の位置は高くその受光率は非常に高かったので階級間での重量生長率の差はこの受光率の差によると推論した. この推論をたしかにするためヒマワリの生長に対する光要因の影響を庇陰格子を使って調べたら，庇陰度の増加とともに重量生長は急激に低下し，伸長生長は極端な庇陰の場合をのぞき大差なかった. また，実測された生産機能と，観測された光・温度要因とを結びつけて算出した重量生長は生長の実測から得られた値と一致して小個体ほど非常に小さかった. これらのことから，群落内における同種間競争において光要因が一つの決定的役割を果すことを確証した.
(東京大学理学部植物学教室)

Bot. Mag. Tokyo, **73**: 310–316 (July 25, 1960)

Electron-microscopical Study on Fine Structures of Diatom Frustules. XVIII

by Haruo OKUNO*

Received February 15, 1960

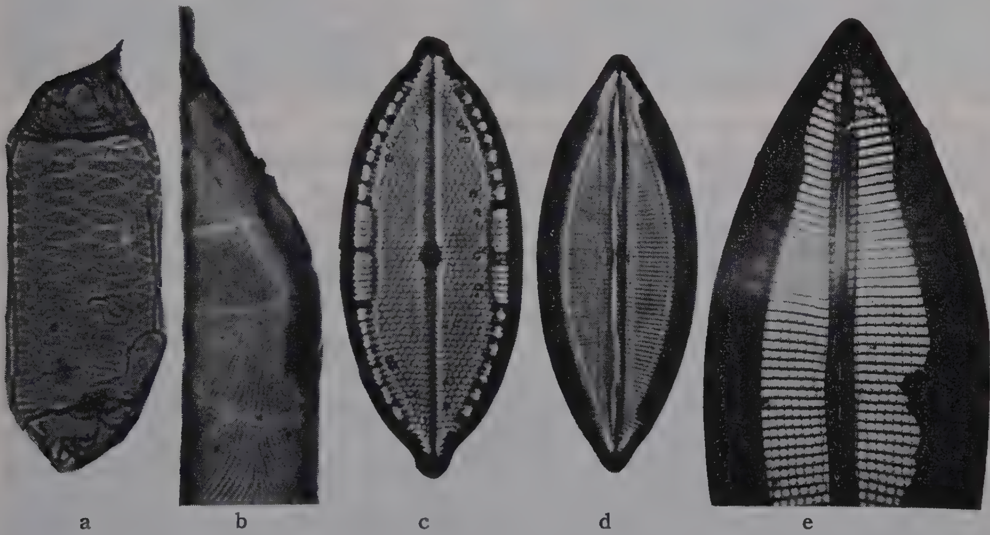
Rhizosolenia alata Brightwell f. **indica** (Peragallo) Ostenfeld (Text-fig. 1a; Pl. I, fig. 1), Hustedt, Kieselalg. **1**: 602, fig. 346 (1930); Desikachary, Mikrosk. **9**: 172, figs. 10–14 (1954).

Cells much larger than the type species. In the present specimen, the frustules about 110μ in diameter and 300μ in length. Intercalary band scale-like, about 40$\times$ 12μ, arranged in longitudinal rows. In shape and number of rows of the intercalary bands, this form akin to *Rhiz. Temperei* and *Rhiz. Castracanei*, but distinctly differs from them by its tubelike, more or less curved oblique setae. Frustule pores on calyptrae and intercalary bands are locular. Loculi on the calyptra, usually round or elliptical, arranged in longer and shorter longitudinal rows about 35–42 in 10μ. In each row, the loculi about 30–36 in 10μ. In the present stereoscopic observation, it was ascertained that the loculi are opened inwards directly into the cell interior and closed outwards by the sieve membranes (the same is in the intercalary bands). The outer sieve membrane has 1–3 irregularly arranged round sieve pores about 40–50mμ in diameter. The inner membrane rudimental, or may be absent. The interlocular space, which is light microscopically smooth, shows super-fine spongy porous structure. Loculi on the intercalary band usually regular or non-regular hexagonal (in those surrounding the interlocular pore, often heptagonal), about 30–36 in 10μ, arranged in different quincunxes on different parts of the band; diameter about 250–400mμ. In the present stereoscopic observation, it was elucidated that the loculus is closed outwards by a sieve membrane and half closed inwards by an inner membrane with a large central opening. (Desikachary described the structure of the loculi of this form as follows: "The areolae are partially open to the outside and on the inside is the sieve membrane."[1]) This description based upon his non-stereoscopic observation is quite contrary to my present stereoscopic observation, and is not correct. His misconception of the position of elements of loculi, I suppose, was caused unavoidably by his non-stereoscopic microscopy. The outer sieve membrane very thin, seems to have six marginal sieve pores (30–40mμ in diameter) at the six corners, and in addition to these, there is one or two sieve pores or poroids in the middle of the membrane which can be seen more clearly than the marginal ones through the opening of the inner membrane. Inner membrane of the loculus is apparently spongy porous, and is provided with a large round central opening about 150–250mμ in diameter. Details of the lateral membrane of the loculus could not be revealed in the present research, and it has not been possible to find if there is any pass pore on it. The depth of the loculus could not be accurately measured, but judging from the stereoscopic image, it may be concluded that the depth is nearly equal to the diameter. Interlocular pores, about 30–50mμ in diameter (the rudimentary loculi; Desikachary's "canal pore"), are scattered about on the entire area of the intercalary band, and do not have any definite arrangement of regularity in the distance between each

* Botanical Laboratory, Kyoto University of Industrial Arts and Textile Fibers, Kitaku, Kyoto, Japan.

other as mentioned by Desikachary[1]). They are usually rectangular, and open on the inside by a rectangular opening and closed on the outside by a sieve membrane with a linear (?) sieve pore. Fine structure of the loculus of intercalary bands of the present form coincides, in its fundamental structure, with that of the type species[2]). On the other hand, f. *inermis*[2]) and a doubtful form[1]), respectively show quite different structure in their sieve membranes. In f. *inermis* the sieve membrane has two parallel lens-shaped sieve pores (correspond to Hendey's frustule structure No. 8[3])), and the doubtful form has no distinct sieve pores but minute poroids (Desikachary, fig. 21). Generally speaking, such a non-coincidence of fine structure between different forms of the same species is rather exceptional, and further detailed research of these forms is expected.

Habitat: Marine plankton. Harimanada, Seto Inland Sea, Hyôgo Prefecture (Okuno, No. m1324. Aug. 1958).



Text-fig. 1. a, *Rhizosolenia alata* f. *indica* (×200). b, *Rhiz. imbricata* var. *Shrubsolei* (×1000). c, *Mastogloia angulata* (×800). d, e, *Mast. apiculata* (d, ×1000. e, ×2000). (a–d, Light micrographs. e, Electron micrograph.)

Rhizosolenia imbricata Brightwell var. **Shrubsolei** (Cleve) Schröder (Text-figs. 1b, 2A; Pl. I, fig. 2), Okuno, Bot. Mag. Tokyo, **70**: 104, pl. 1, figs. 4a, b (1957).

I already reported in my previous paper above mentioned, some of the electron microscopical fine structures of this variety observed by the non-stereoscopic electron microscopy, and further reviewed the Desikachary's electron micrographs which he published under the name of this variety and of *Rhiz.* sp.[4]) In the present stereoscopic observation, the following facts were ascertained or more correctly found: Loculi are closed on the outside by the sieve membranes and open almost fully on the inside. The ratio between the length, breadth and depth of the loculus is about 2–2.5:1:0.7–0.8. In loculi along the longitudinal axis of the intercalary band, the length and breadth are usually much reduced, and the directions of the diagonal sieve pores are irregular. The diagonal sieve pore is usually a single linear slit, sometimes it is divided into several minute oblong pores, or it is closed outwards by a thin membrane. The sieve membrane is somewhat thick on its outer margin and from there becomes thin gradually to the border of the sieve pore, and sometimes

has scattered minute granules impenetrable to the electron beam. Such granules were sometimes found also on the limb of the intercalary band. The sieve membrane and the limb of the intercalary band distinctly show the fine spongy porous structure.

Habitat: Marine plankton. East China Sea (32–54N: 124–12E) (Okuno, No. m932, Sept. 1953. Collected by H. Maeda).

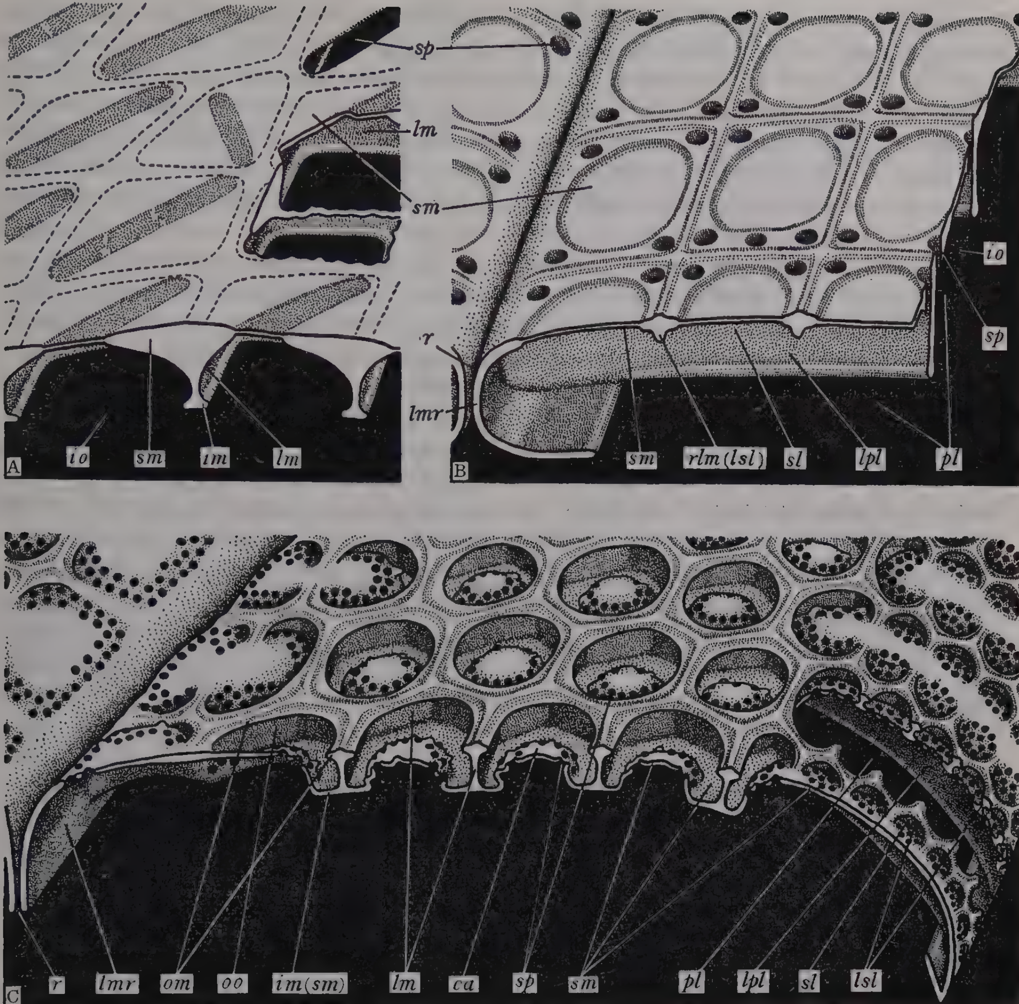
Mastogloia angulata Lewis (Text-figs. 1c, 2C; Pl. I, fig. 3), Okuno, Bot. Mag. Tokyo, **70**: 222, pl. 7, figs. 2a–c (1957).

In my previous paper above mentioned, I reported some of the fine structure of this species observed by the electron non-stereomicroscopy. In the present stereomicroscopy, further details of the three dimensional character of the fine structure of the frustule wall was much clearly revealed, and accordingly some of my previous description of the fine structure must be revised here. The present research reveals the following structure: Loculi in the central part of the valve, usually non-regular hexagonal, arranged in slightly radiating rows, about 9–11 in 10μ, and in each row loculi about 8–10 in 10μ. Loculus opened outwards and closed inwards by a sieve membrane. Ratio between the diameter and the depth of a loculus seems to be nearly equal to 2:1. The outer membrane narrow, marginal (about 10–15mμ broad), leaving a large round opening about 70–80mμ in diameter. The inner membrane is divided into two parts, the central dome-shaped area projected outwards (diameter about 400–600mμ) and the marginal flat area. The central area on its margin with a circular row of round sieve pores about 20–30mμ in diameter, and sometimes scattered with several pores inside the circle. The lateral membrane of the loculus seems to be non-porous. Transverse row of the hexagonal loculi ends on the margin of the valve in a double row of small incomplete loculi (the secondary loculi[5]) about 2 in 1μ, and each double row represents a transversely elongated marginal compound oculus (the primary loculus[5])). In the secondary loculus, the inner sieve membrane does not seem to be projected centrally, and has scattered round sieve pores over the whole area. Outer membrane of the secondary loculi indistinct, or may be absent. The marginal primary loculus seems to have a little higher lateral membrane than the central hexagonal loculi.

Habitat: Marine, littoral. Minato, Seidan-chô, Awaji, Hyôgo Prefecture (Okuno, No. m1020. Aug. 1955).

Mastogloia apiculata W. Smith (Text-figs. 1d, e. 2B; Pl. I, fig. 4), Synop. Brit. Diat. **2**: 65, pl. 62, fig. 387 (1956); Hustedt, Kieselalg. **2**: 515, fig. 946 (1933); Mills, Index Diat.: 898 (1933); Cleve-Euler, K. V. A. Handl. **4**, no. 5: 58 (1953).

Valves elliptic-lanceolate, with more of less produced ends. Length 47–60 (40–90)μ; breadth 22 (16–24)μ. Marginal loculi about 2 μ broad, quadrate, 8–9 in 10 μ, inner borders flattened, forming a band, ending near the ends of valve. Transverse rows of loculi 15–16 (15–20) in 10μ, slightly radiate; loculi 18–20 in 10μ, forming undulating longitudinal rows. The present electron stereomicroscopy reveals the following structure: Valve surface concave, submarginally highest, falls down gradually towards the raphe and abruptly towards the margin. Valve wall, doubly locular. Primary loculus which corresponds to the transverse row of areolae or alveoli of the light microscope image, is transversely elongated, and fully opened towards inside and closed towards outside by a lamina of secondary loculi. Lateral wall of the primary loculus is transverse, very thin, slightly thickened at its inner margin, and apparently hyaline, but it is finely spongy porous as the other parts of the valve wall. The transverse lateral membrane may be slightly projected outwards as a low lamella. Secondary loculus rectangular, about 500×650 mμ, 18–20 in 10 μ, arranged



Text-fig. 2. Diagrams of fine structure of diatom valves reconstructed from electron stereomicrographs. A, *Rhizosolenia imbricata* var. *Shrubsolei*. B, *Mastogloia apiculata*. C, *Mast. angulata*. (*ca*, Central area of inner sieve membrane. *im*, Inner membrane. *io*, Inner opening. *lm*, Lateral membrane. *lmr*, Lateral membrane of raphe. *lpl*, Lateral membrane of primary loculus. *lsl*, Lateral membrane of secondary loculus. *om*, Outer membrane. *oo*, Outer opening. *pl*, Primary loculus. *r*, Raphe. *rlm*, Rudimentary lateral membrane. *sl*, Secondary loculus. *sm*, Sieve membrane. *sp*, Sieve pore.)

in a transverse single row in each primary loculus, and closed towards outside by a sieve membrane and open freely on the inside into the primary loculus. The outer sieve membrane, with a thin round central area about 300–400 mμ in diameter and the thick margin provided with four round sieve pores (60–70 mμ in diameter) at the four angles, and in addition to these, sometimes, there is a sieve pore inside the middle of the transverse margin of the membrane. Central area of the sieve membrane very thin (often destroyed during preparation), with scattered fine pores or poroids and distinctly spongy porous. The longitudinal lateral membranes of the secondary loculi very low, slightly projected inwards, and the inner cover membrane

absent. Axial area of this species which hitherto misunderstood by its light microscopical image to be a thickened border of the raphe or a sort of rib run along the raphe, is elucidated in the present research, not to be a thickening or a rib, but to be a doubly laminar part along the raphe. The inner border of the outer membrane of the primary loculus turn inwards along the raphe and then bent back sidewards to run parallel to the outer membrane for about 1 μ. The part turned inwards which represents the side wall of the raphe is apparently smooth. The side turned, parallel running part is smooth and transparent enough for the fine structure of the outer membrane to be seen through it. Such a parallel running inner membrane of the valve on both sides of the raphe was also found by me in the raphe-valve of *Cocconeis ceticola*[7]). The electron stereomicrographs of *Achnanthes hungarica*[8]), *Ach. longipes*[9]), and *Pinnularia appendiculata*[10]) show much thicker side wall of the raphe, and in such a case the axial area can be described being ribbed. From the present observation, it may be stated that such a doubly laminar structure of the axial area is to a certain extent common to the locular raphe-valve. In the light microscopy, the raphe of this species was considered to be a single straight fissure on its whole length and described being "straight", but in the present electron microscopy, it was elucidated that the raphe is a vertical slit of about 50 mμ breadth, and at least in the middle one-third is folded or may be divided into the two, straight and acurate fissures by a thin oblique septum. Most of the diatoms hitherto researched with the electron microscope showed the straight raphe, and only in a few species, the folded raphe was found. In electron microscopy, the straight raphe was found in *Achnanthes lanceolata*[11,12]), *Amphipleura rutilans*[13]), *Amphora ovalis* var. *pediculus*[14]), *Cocconeis ceticola*[7]), *Cocc. scutellum*[15]), var. *parva*[16]), *Cocc. stauroneiformis*[17]), *Diploneis chersonensis* (Okuno, unpublished), *Mastogloia Smithii*[18]), *Navicula pelliculosa*[19-21]), *Nav. pygmaea*[22]), *Nav. tripartita*[23]), *Nav.* sp.[20]), *Phaeodactylum tricornutum*[24]), *Rhoicosphenia curvata*[25]). The folded raphe was found in *Mastogloia Braunii*[26]) and *Navicula Trompii*[27]), and the canal raphe was found in *Epithemia sorex*[28]), *Nitzschia* sp.[29]), *Rhopalodia musculus*[30]), *Surirella gemma*[31]).

Habitat: Marine littoral. Seidan-chô, Awaji, Hyôgo Prefecture (Okuno, No. m1020. Aug. 1955).

Summary

Fine structure of the frustules of four marine diatoms are described presenting their electron stereomicrographs.

Rhizosolenia alata f. *indica*: Both loculi on calyptra and intercalary band are closed outwards and opened inwards. Loculi on the calyptra round or elliptical, arranged in long and short longitudinal rows. The outer sieve membrane has 1-3 irregularly arranged sieve pores about 40-50 mμ in diameter. Loculi on the intercalary band hexagonal, about 250-400 mμ in diameter, in quincunxes. The outer sieve membrane has six marginal sieve pores each about 30-40 mμ in diameter. The inner membrane has a central opening about 150-250 mμ in diameter.

Rhizosolenia imbricata var. *Shrubsolei*: Loculi parallelogramatic on the intercalarly band, closed outwards by a sieve membrane and almost fully opened inwards. The outer sieve membrane has a long diagonal sieve pore, which is often divided into several minute pores or closed by a delicate membrane.

Mastogloia angulata: Loculi hexagonal in the central part of the valve, opened utwards and closed inwards. The inner sieve membrane has a central dome-shaped

projection with a circular row of sieve pores each about 20–30 mμ in diameter. On the margin of the valve, each row of the hexagonal loculi ends in a double row of small incomplete loculi which represent the secondary loculi.

Mastogloia apiculata: Primary loculi transverse, groove-shaped, each closed outwards by a row of secondary loculi, and opened freely inwards. Lateral membranes of the primary loculi are spongy porous. Secondary loculi rectangular, about 500× 550 mμ, each closed outwards by a sieve membrane with four sieve pores at four angles.

The electron stereomicrographs in plate I and II of my previous paper (Bot. Mag. Tokyo, **72**: 61–67) were presented by the halftone black and white printing, and when viewed with a magnifying stereoscope, the meshed image of printing is seen disturbing the correct understanding of the fine structure of the diatom frustules. The fine structure of frustules shown in such micrographs will be correctly understood by viewing them with naked eyes. The method of such viewing was described in my previous paper.

References

1) Desikachary, T. V , Mikrosk. **9**: 172 (1954). 2) Okuno, H., Journ. Jap. Bot. **27**: 353 (1952). 3) Hendey, N. I., Journ. Quekett Microsc. Cl. Ser. 4, **5**: 160 (1959). 4) Desikachary, T. V., Mikrosk. **9**: 170 (1954). 5) Okuno, H., Rev. Cyt. et Biol. Végét. **15**: 238 (1954). 6) Desikachary, T. V., Amer. Journ. Bot. **41**: 616 (1954). 7) Okuno, H., Journ. Jap. Bot. **29**: 271 (1954). 8) Helmcke, J.–G., and Krieger, W., Diat. Elektr. Bild, 1, pl. 51 (1953). 9) Okuno, H., Bot. Mag. Tokyo, **72**: 66 (1959). 10) Helmcke, J.-G., and Krieger, W., Diat. Elektr. Bild, 1, pl. 74 (1953). 11) ——, ibid. 1, pl. 53 (1953). 12) ——, Zeits. Wiss. Mikrosk. Techn. **60**: 197 (1951). 13) Kolbe, R. W., Ber, Deut. Bot. Ges. **61**: 94 (1943). 14) Helmcke, J.–G., and Krieger, W., Diat. Elektr. Bild, 2, pl. 181 (1954). 15) ——, ibid. 1, pl. 47 (1953). 16) Okuno, H., Bot. Mag. Tokyo, **70**: 221 (1957). 17) ——, ibid. **72**: 217 (1959). 18) Helmcke, J.–G., and Krieger, W., Diat. Elektr. Bild, 2, pl. 160 (1954). 19) Kolbe, R. W., Ber. Deut. Bot. Ges. **61**: 93 (1943). 20) Lewin, J. C., Journ. Gen. Microbiol. **9**: 305 (1953). 21) ——, Canad. Journ. Microbiol. **3**: 427 (1957). 22) Helmcke, J.-G., and Krieger, W., Diat. Elektr. Bild, 1, pl. 71 (1953) 23) ——, ibid. 2, pl. 176 (1954). 24) Lewin, J. C., Journ. Gen. Microbiol. **18**: 427 (1958). 25) Helmcke, J.–G., and Krieger, W., Diat. Elektr. Bild, 1, pl. 56 (1953). 26) ——, ibid. 1, pl. 57 (1953). 27) ——, ibid. 2, pl. 175 (1954). 28) ——, ibid. 1, pl. 80 (1953). 29) Kolbe, R. W., Sv. Bot. Tids. **45**: 642 (1951). 30) Helmcke, J.–G., and Krieger, W., Diat. Elektr. Bild, 1, pl. 83 (1953). 31) ——, Verh. Deut. Zool. Ges. Wilhelmshaven, p. 439 (1951).

摘　　　要

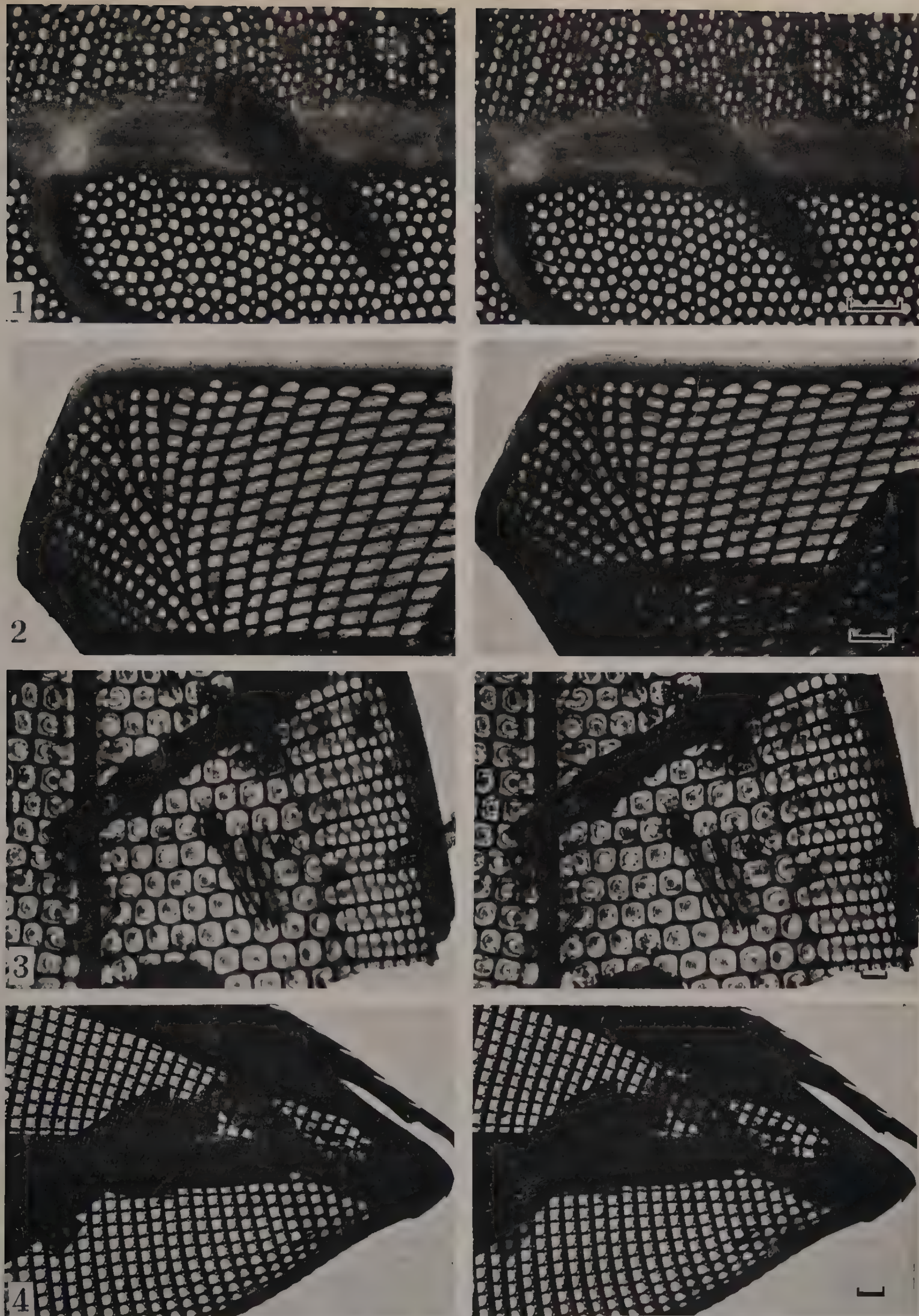
奥野春雄: 電子顕微鏡による珪藻殻微細構造の研究 XVIII

つぎの4種類の海産現生種珪藻殻の電子顕微鏡的立体微細構造について記した.

Rhizosolenia alata f. *indica*: 蓋殻 (Calyptra) の孔房は円形または楕円形で縦列にならび, 中間帯 (Intercalary band) の孔房は六角形で, 60° に交わる3直線列にならぶ. 孔房は蓋殻, 中間帯のいずれのものも外閉内開型で, その外膜は篩孔をもつ篩膜となり, 内膜はせまく, 中央に大きい内孔をもつ. *Rhizosolenia imbricata* var. *Shrubsolei*: 中間帯の孔房は前種と同じく外閉内開型であり, 外膜には対角線の方向に線形の篩孔が1つある. 篩孔はときに数個の小孔に分たれている場合もある. 外膜は篩孔近くでうすく, 孔房周辺に向ってやや厚さを増す. 篩膜に電子線の不透な不定形小粒子の散在する場合も見られた.

内膜は不顕著である．*Mastogloia angulata*: 立体写真により孔房が外開内閉型であることが判明した．著者の前報文（植雑，**70**: 222）では平面写真から推定して孔房が外閉内開型であると記したが，それは誤りであった．孔房は六角形を基本形とし，横列にならび，その外膜はきわめてせまく，孔房は外へ広く開く．内膜は中央部がドーム状に外方へ隆起し，そのふちに環状にならんだ篩孔群がある．珪殻の左右縁では各孔房列はさらに小さい2列の孔房群に分れている．これら2列ずつの小孔房は共通の横側膜をもつ集合孔房となる．*Mastogloia apiculata*: 孔房は外閉内開型で，しかも二層孔房となる．一次孔房（大孔房）は横走する溝状孔房で，その外側は1列の二次孔房（小孔房）層で閉じ，内側は全開する．二次孔房は光学顕微鏡的に Areolae または Alveoli と記載されたもので，その外側は四隅に1個ずつの篩孔をもつ篩膜でとざされ，内側は内膜なく一次孔房に全開する．

第17報（植雑，**72**: 61–67）では電顕写真図版（Pls. I, II）を網目写真版としたので，ルーペ式立体鏡で見た場合に印刷網目が見え，珪殻微細構造とまぎらわしい結果となった．それらは裸眼による方法で見て，微細構造を理解されたい．（京都工芸繊維大学繊維学部植物学研究室）



Pairs of electron stereomicrographs. Fig. 1, *Rhizosolenia alata* f. *indica* (upper part, the calyptra; lower part, the intercalary band). 2, *Rhiz. imbricata* var. *Shrubsolei* (intercalary band). 3, *Mastogloia angulata*. 4, *Mast. apiculata*. Scales: $1\,\mu$. (When viewed through the stereoscope with parallel visual axis, Figures 1–3 show the inside view, and Figure 4 shows the outside view.)

Bot. Mag. Tokyo **37** : 317-325 (July 25, 1960)

Micrococcus glutamicus の細胞学的研究

第4報　菌形態におよぼす biotin の影響について

板　垣　史　郎*

Shiro ITAGAKI*: Cytological Studies on *Micrococcus glutamicus*.
Part IV. The Effect of Biotin on the Morphological
Character of *Micrococcus glutamicus*.

1959 年 12 月 15 日受付

緒　　言

Micrococcus glutamicus は，biotin を微量(2.5 γ/l) 添加した合成培地中での培養経過において，形態をかなり変化させ，一見不整桿菌形態を示すが，これは必須成分である biotion が欠乏しているために完全分裂がおこなわれず，多細胞体を形成するものであることを知りすでに報告した[1]).

また，培養のある時期にきわめて鮮明な metachromasy をしめす極顆粒が形成され，このものの本体はおそらくは酸不溶性のポリリン酸であろうと述べた[2]). この metachromatic granule (以下 m. granule と略) は，培地成分，培養時期などによって特長的な消長をしめす.

今回は biotin と菌形態の関係を中心に観察し，*M. glutamicus* に属する数株の間にもそれぞれいくらかの性質の差を認めた.

実験材料および実験方法

使用菌株および培地

Micrococcus glutamicus 516, 534, 541, 560, 582, 588 および 591 の7株を用いた.

使用培地は (第1報)[1]) に記載した glucose bouillon および合成培地を用いた. ただし，合成培地の biotin 量は，各実験項目の所に記載したように，種々の濃度に添加し，また目的によって酵母エキスおよび corn steep liquor を添加した.

培養方法

各培地を 250 ml 三角フラスコに 50 ml ずつ加え，rotary shaker にて 28° で培養した.

glucose bouillon 前培養 5 ml を遠沈集菌し，生理食塩水にて洗滌後，さらに所定の試験培地に懸濁し，これをそのまま試験培地に移した. すなわち，前培養の培地成分が試験培地に混入することを防ぎ，植菌量を 10% とした. かくして培養しつつ適宜の時間に適当量の試料を採取し，菌体量，pH を測定し，あわせて methylene blue 染色による菌形，m. granule 形成，細胞膜染色による septa の形成および菌外形の観察をおこなった.

結　　果

1. biotin 含量の影響

——生育，pH，septa 形成および m. granule の形成について——

基本合成培地に対し，biotin を 0〜500 γ/l (9段階) 添加した (第1表).

本実験は，培地組成変化を菌自体によるもの以外可及的に避けるためと，培養途次の pH 変化をみるため，pH の調節をおこなわなかった. したがって，菌体の生育にともなう pH の変化自身に生育を抑制阻害されることも考えられるので，菌生育の条件としてはやや不良である. このため，生長曲線も乱れ，やや不安定な型をしめすが，一般に biotin が 5 γ/l 以下では生育はやや不良である.

(第1表)にしめす生育は，上に述べたような条件ではかなり再現性が強く，*M. glutamicus* 7株の性質の一端をしめすものといえよう. また，m. granule の形成もかなり安定したようすをしめすが，その形成を数値的に表現することは相当に困難である. Mudd ら[3]) は *Corynebacterium diphtheriae* の同様顆粒を表わすのに Index of Metachromasy

* 協和醱酵工業株式会社東京研究所 Tokyo Research Laboratory, The Kyowa Fermentation Industry Co. Ltd.

第1表 *M. glutamicus* に対する biotin 量の影響——生育，pH 変化，septa および m. granule の形成——

Strain	γ/l Biotin	Growth mg/ml				Septa				Metachromatic granule			
		0 day	1	2	3	0 day	1	2	3	0 day	1	2	3
	0	0.16	1.6	1.9	1.7	0-1 1	1-3 5	1-4 6	1-4 9	±	±	−	−
	0.1	0.16	2.1	2.5	2.2	0-1 1	1-3 4	1-3 5	1-3 5	±	±	±	−
	0.25	0.16	2.6	3.1	2.1	0-1 1	1-3 5	2-4 7	1-3 6	±	±	−	−
	0.5	0.16	3.9			0-1 1	1-3 5	1-3 5	1-3 3	±	±	−	−
516	1.0	0.16	4.8	6.5		0-1 1	1-3 4	1-3 5	1-3 4	±	±	±	−
	5.0	0.28	8.2	9.8	8.0	0-1 5	0-1 5	0-1 1	0-1 5	+	−	±	++++
	25.0	0.28	8.8	10.2	7.8	0-1 5	0-1 1	0-1 1	0-1 4	+	−	−	++++
	100.0	0.28	7.8	10.3	7.2	0-1 5	0-1 1	0-1 1	0-1 3	+	−	±	++++
	500.0	0.28	9.8	10.5	7.2	0-1 5	0-1 1	0-1 1	0-1 3	+	−	−	++++
	0	0.25	1.8	1.7	1.7	0-1 2	1-3 4	1-3 5	1-3 5	−	++±	+	−
	0.1	0.25	1.9	2.0		0-1 2	1-3 5	1-3 5	1-3 3	−	+++	±	−
	0.25	0.25	2.4	2.4		0-1 2	1-3 5	1-3 4	1-3 5	−	+++	+±	+
	0.5	0.25	2.6	3.1		0-1 2	1-3 5	1-3 4	1-3 6	−	+++	++	−
534	1.0	0.25	3.3	3.6	4.0	0-1 2	1-3 5	1-3 5	1-3 5	−	++±	+	+
	5.0	0.33	8.2	10.0	9.2	0-1 2	0-2 3	0-1 3	1-3 4	±	−	−	+++
	25.0	0.33	10.0	9.4	9.0	0-1 2	0-2 2	0-1 2	1-3 3	±	−	−	++±
	100.0	0.33	10.3	9.4	9.8	0-1 2	0-1 1	0-1 1	1-3 3	±	−	+	++
	500.0	0.33	10.0	9.4	8.8	0-1 2	0-1 1	0-1 1	1-3 3	±	−	−	+±
	0	0.13	1.9	1.8		0-1 2	2-4 6	1-3 6	1-3 6	±	±	−	−
	0.1	0.13	2.3	2.9		0-1 2	1-3 5	1-3 4	1-3 4	±	±	±	−
	0.25	0.13	2.8	3.0		0-1 2	1-3 5	1-3 6	1-3 5	±	±	−	−
	0.5	0.13	3.8	4.1		0-1 2	1-3 4	1-3 6	1-3 4	±	±	−	−
541	1.0	0.13	5.1	6.0		0-1 2	1-3 4	1-3 5	1-3 4	±	±	−	−
	5.0	0.29	9.4	8.2	7.8	0-1 2	0-1 2	0-1 2	1-3 3	±	−	−	±
	25.0	0.29	10.5	8.3	7.8	0-1 2	0-1 2	0-1 3	1-3 3	±	−	−	±
	100.0	0.29	11.0	8.4	8.2	0-1 2	0-1 1	0-1 3	1-3 3	±	−	+	−
	500.0	0.29	10.6	8.2	8.0	0-1 2	0-1 3	0-1 1	1-3 4	±	−	±	−
	0	0.20	2.2	2.7	3.0	0-1 1	1-3 5	1-3 7	1-3 4	+	+++	+±	−
	0.1	0.20	2.4	2.4	3.1	0-1 1	1-3 5	1-3 5	1-3 4	+	+++	±	−
	0.25	0.20	2.7	3.1	3.0	0-1 1	1-3 4	1-3 6	1-3 5	+	++±	−	−
	0.5	0.20	3.7	4.5	4.1	0-1 1	1-3 6	1-3 6	1-3 3	+	++±	−	−
560	1.0	0.20	4.4	4.5	4.9	0-1 1	1-3 3	1-3 3	1-3 3	+	±	−	−
	5.0	0.40	5.2	5.3	6.8	0-1 1	0-1 2	0-1 1	1-3 3	+	−	−	+++
	25.0	0.40	5.6	4.7	6.2	0-1 1	0-1 4	0-1 1	1-3 3	+	−	−	+++
	100.0	0.40	5.2	4.8	7.2	0-1 1	0-1 2	0-1 1	1-3 3	+	−	−	+++
	500.0	0.40	3.9	5.7	6.4	0-1 1	0-1 1	0-1 1	1-3 3	+	−	−	+++
	0	0.16	2.3	2.1	2.0	0-1 3	1-3 7	1-3 5	1-3 5	±	++±	++±	+±
	0.1	0.16	2.4	2.5	2.4	0-1 3	1-3 5	1-3 5	1-3 4	±	+++	++	±
	0.25	0.16	3.0	3.0	3.1	0-1 3	1-3 5	1-3 7	1-3 6	±	+++	+±	−
	0.5	0.16	4.5	4.3	4.8	0-1 3	1-3 4	1-3 5	1-3 4	±	++	−	−
582	1.0	0.16	5.1	6.8	5.0	0-1 3	1-3 4	1-3 3	1-3 3	±	+++	−	−
	5.0	0.31	6.4	7.2	9.8	0-1 1	0-1 2	0-1 1	0-1 3	±	−	−	++
	25.0	0.31	7.4	8.3	9.8	0-1 1	0-1 1	0-1 1	0-1 4	±	−	−	+++
	100.0	0.31	7.0	9.6	9.8	0-1 1	0-1 2	0-1 3	0-1 3	±	−	−	+
	500.0	0.31	7.0	9.2	10.5	0-1 1	0-1 1	0-1 2	1-2 3	±	−	−	+
	0	0.20	2.7	3.1	3.3	0-1 1	1-3 5	1-3 5	1-3 4	±	±	−	−
	0.1	0.20	3.2	3.9	3.7	0-1 1	1-3 4	1-3 4	1-3 3	±	±	−	−
	0.25	0.20	3.6	4.1	4.3	0-1 1	1-3 3	1-3 4	1-3 5	±	−	−	−
	0.5	0.20	4.6	5.3	5.1	0-1 1	1-3 3	1-3 5	1-3 4	±	±	−	−
588	1.0	0.20	5.3	6.3	6.5	0-1 1	1-3 3	1-3 5	1-3 4	±	±	−	−
	5.0	0.40	8.4	7.6	7.8	0-1 1	0-1 2	0-1 2	1-3 5	±	−	−	++++
	25.0	0.40	9.0	8.3	7.6	0-1 1	0-1 3	0-1 1	1-3 3	±	−	−	+++
	100.0	0.40	8.8	8.3	9.0	0-1 1	0-1 2	0-1 1	1-3 3	±	−	−	+
	500.0	0.40	8.8	7.6	8.3	0-1 1	0-1 2	0-1 3	1-3 5	±	−	−	++
	0	0.20	1.8	1.7	1.7	0-1 1	1-3 5	1-3 4	0-2 3	±	++++	−	−
	0.1	0.20	1.8	1.5	1.7	0-1 1	1-3 4	1-3 3	0-2 3	±	++++	−	−
	0.25	0.20	1.9	1.7	1.9	0-1 1	1-3 3	1-3 6	0-2 3	±	++++	±	−
	0.5	0.20	2.1	1.5	2.2	0-1 1	1-3 3	1-3 4	0-2 4	±	++++	+±	+
591	1.0	0.20	2.3	2.7	3.0	0-1 1	1-3 3	1-3 3	0-2 4	±	++++	±	−
	5.0	0.30	2.6	1.0	0.9	0-1 1	1-3 5	0-3 3	1-2 3	±	+++	−	−
	25.0	0.30	3.4	1.0	3.8	0-1 1	1-3 3	0-3 3	1-2 3	±	++	−	−
	100.0	0.30	5.4	5.7	6.1	0-1 1	1-3 3	0-3 4	1-2 4	±	++	−	−
	500.0	0.30	2.9	8.2	10.0	0-1 1	1-3 5	0-3 3	0-1 2	±	+++	−	++

(I. M.) という言葉を用い，数量的に表現しているが，さほど意味があるとも考えられない．著者は，この顆粒が acid insoluble polyphosphate より成ることを認めて報告したが，この方法により燐酸区分の定量をおこない，m. granule の多寡を比較することは可能であるが，定量法はかなり複雑であり，多数の試料については不適当である．そこで概略の値として，ほとんどの菌体に認められない場合を－とし，ほとんど全菌体に 2～3 個認められる場合を+++++とし，その間の形成度合をおおむね10段階にわけて表示した．ただし，形成された m. granule も非常に小さかったり，大きかったりする場合も多いので，このような場合には適当に勘案した．

本菌の合成培地における菌形態の変化は，すでに述べたように biotin の欠乏にもとづく不完全分裂の結果多細胞体が形成されるのであるが，この形態変化をしめす一つの基準として septa の数を観察した．

すなわち，第1表の septa の項の前項には population を形成する大部分の菌体で認められる septa の数を，後項には塗沫染色標本中で，菌体が重なり合わず，明瞭な観察を行ない得る視野につき充分多数の菌体を観察した範囲内で，見掛け上の1細胞が有する最も多数の septa 数をしめした．この数の septa をもつ細胞は，検鏡の場合わずか1個のみ認められた場合もあり，多数の菌体において認められた場合もあるので，この数値をもって一概に菌の異常形態の目安とすることにはある程度危険がともなうが，(第1表)のごとく，biotin 含量が多い場合は前項の数値も，後項の数値も小さくなり，明らかに多細胞体の形成は biotin の欠乏によることをしめすといえよう．

2. *M. glutamicus* 541 の biotin 含量による影響

——特に寒天培地上における菌形態について——

541 株は 534 株ともに *M. glutamicus* の代表的な株であるが，通常の組成の合成培地中ではあまり m. granule を形成せず，むしろ glucose bouillon 中で形成しやすいことは，すでに報告したが，この菌による septa の形成，m. granule の形成を精細に調べた．特に寒天培地上での生育の場合について観察した．

biotin 量を 0～1000 γ/l まで 14 段階にわけて添加した．この結果をしめすものが（第2表）である．生長は実験1における（第1表）と同一の形をしめす．pH の変動，septa の形成も同じ結果であった．この3点の性質には再現性がみられる．ただし，m. granule の形成は，（第1表）と異なり，biotin 10～250 γ/l，2日培養にいちじるしい形成をみたが，3日目にはほとんど消失している．その代りに 5 γ/l のものに形成がみられる．このように 541 は m. granule 形成に関してはやや不安定である．

今回の実験では，541 を用いて寒天培地上の形態もあわせて観察した．培地は基本合成培地に biotin を 0～1300 γ/l の 14 段階にわけて添加し，さらに 2％の割に寒天を加えた．各段階共内径約 25 mm の試験管に 15 ml ずつ加えて斜面とし，一定量の植

第2表 *M. glutamicus* 541 の biotin 含量による影響

γ/l Biotin	Growth mg/ml					pH				Septa			Metachromatic granule			
	0 day	1	2	3	4	1 day	2	3	4	1 day	2	3	0 day	1	2	3
0	0.3	4.6	4.8	4.8	5.2	7.6	5.0	5.6	5.4	5	4	4	+	++	−	−
0.1	0.3	4.2	4.6	4.5		7.6	5.4	5.6		4	3	5	+	++	−	−
0.25	0.3	4.7	4.8	5.3		7.4	5.2	5.4		6	8	5	+	++	−	−
0.5	0.3	5.3	5.5	5.6	6.0	6.8	5.6	5.6	5.2	5	6	5	+	++	−	−
1.0	0.3	7.2	7.2	8.2		5.0	5.6	5.6		4	4	5	+	++	−	−
2.5	0.3	8.2	8.2	8.2	9.0	5.4	5.8	6.8	8.0	3	3	3	+	±	−	+
5.0	0.3	9.0	9.0	9.0	8.4	5.0	5.6	8.0	8.0	3	2	4	+	−	−	+++
10.0	0.3	11.0	10.0	9.0	8.4	5.0	7.5	8.0	7.4	3	3	3	+	−	++++	+
25.0	0.3	11.5	10.0	9.4	8.1	4.8	7.2	8.0	7.8	3	1	4	+	−	++++	±
50.0	0.3	12.5	11.1	9.2	8.4	5.4	7.2	8.2	8.2	1	3	3	+	−	++++	±
100.0	0.3	12.5	11.2	10.5	11.0	5.0	7.6	8.2	8.2	1	4	4	+	−	++++	±
250.0	0.3	12.5	11.1	10.2	10.0	5.4	7.6	8.0	8.2	2	3	3	+	−	+±	−
500.0	0.3	12.0	9.8	10.0	9.8	5.6	7.0	8.0	8.0	2	2	3	+	−	++	−
1000.0	0.3	12.0	9.8	10.0	10.0	5.4	7.2	7.6	8.2	1	3	3	+	−	+	−

菌をおこなった．生長が進行するにつれて培地のpH は低下し，このため培地中の phenol red が黄変する．この色調の変化によって生育の度合もほぼ判明する．

第3表 *M. glutamicus* 541 の biotin 含量による影響，寒天培地上にて生育の場合

γ/l Biotin	Growth mg/ml		Septa		M. granule
	1 day	2	1 day	3	1 day
0	±	±	1-2 7	1-3 10	++
0.13	±	±	1-2 8	1-3 11	++
0.33	±	±	1-2 9	1-3 5	++
0.65	+	+	1-2 9	1-3 7	++
1.3	++	++	1-2 7	1-3 9	±
3.3	++±	++++	0-1 5	1-3 6	+++
6.5	+++	+++++	0-1 5	1-3 5	+++
13.0	+++	+++++	0-1 3	1-3 4	++++
33.0	++++	++++++	0-1 4	1-3 3	++++
65.0	++++	++++++	0 3	1-3 3	++++
130.0	+++++	++++++	0 2	1-3 5	++++
330.0	++++++	++++++	0 3	0-1 3	++++
650.0	+++++++	+++++++	0 1	0-1 3	++++
1300.0	+++++++	+++++++	0 2	0-1 3	++++

かくのごとき実験の結果をしめしたのが(第3表)である．この場合 biotin を 5 γ/l 程度以上添加したものはいちじるしい生長を示し，しかもこの時の菌体にはほとんど septa が認められず，glucose bouillon 中におけるがごとき類楕円形形態をしめす．しかし生育が不良である 3 γ/l 以下では同組成の液体培養に比しいちじるしい不整形態を示し，septa 形成も一段とはげしい．このことは培養3日間を通じて変らない．

一方，m. granule の形成は液体培地の場合に反し規則正しく，生長が良好 (biotin 量が多い) な場合程整一な形成をしめした．

3. biotin の代りに酵母エキスを添加した場合の菌形態

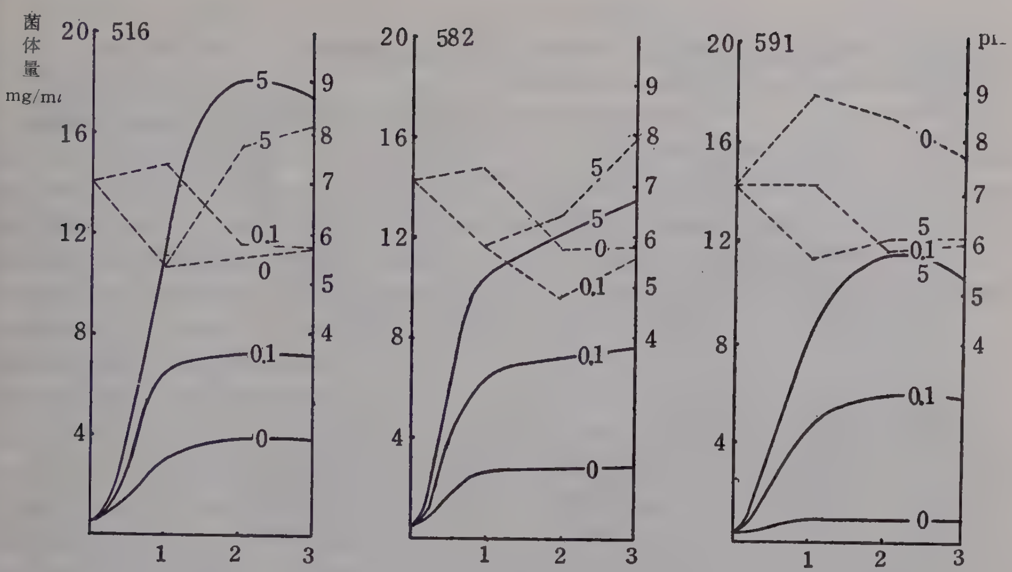
すでに何度ものべたごとく，*M. glutamicus* の菌形態は biotin の量により大きく左右される．そこで biotin に代るものとして酵母エキスを添加して実験をおこなった．使用した酵母エキスは大五栄養化学製粉末酵母エキスである．この酵母エキスの biotin 含量は，bioassay により 625 mγ/g であるから，添加した酵母エキスの量より換算し培地中の biotin 量は 0～31.25 γ/l の7段階となる．使用した菌株は 516, 582 および 591 の3株である．

この結果を（第4表）および（第1図）にしめした．

いうまでもなく，酵母エキス中にはビタミン複合体が豊富に含有されているので，本菌の生育も

第4表 *M. glutamicus* に対する酵母エキス添加量の影響——生育，pH 変化，septa および m. granule の形成——

Strain	Yeast ext. %	γ/l Biotin	Growth mg/ml				Septa				Metachromatic granule			
			initial	1 day	2	3	initial	1 day	2	3	initial	1 day	2	3
516	0	0	0.46	3.1	3.9	3.9	0 1	2-3 6	1-3 5	1-3 5	−	−	−	−
	0.01	0.063	0.46	3.8	4.2	4.8	0 1	2-3 5	1-3 7	1-3 6	−	−	−	−
	0.05	0.313	0.46	4.6	7.2		0 1	2-3 6	1-3 5	1-3 6	−	−	−	−
	0.1	0.625	0.46	6.7	7.2	7.3	0 1	1-2 3	0-2 6	1 4	−	−	−	−
	0.5	3.125	0.46	8.6	10.5	10.8	0 1	0 1	0 3	1 5	−	−	−	±
	1.0	6.25	0.46	11.0	14.0	12.5	0 1	0 1	0 2	1-2 3	−	−	−	±
	5.0	31.25	0.46	11.5	18.0	17.5	0 1	0-1 1	0-2 4	1-1 3	−	−	−	±
582	0	0	0.48	2.9	2.9	3.0	0 1	0-1 3	0-1 3	0-1 2	++	++	−	−
	0.01	0.063	0.48	3.6	3.4	3.6	0 1	1-2 4	1-3 6	1-2 4	++	++	−	−
	0.05	0.313	0.48	5.6	5.8	6.0	0 1	1-3 5	2-3 6	2-3 4	++	+	−	−
	0.1	0.625	0.48	6.8	7.2	7.8	0 1	1-3 3	1-3 3	1-2 3	++	−	−	−
	0.5	3.125	0.48	7.8	9.0	10.5	0 1	0 2	0-1 4	0-1 3	++	−	−	−
	1.0	6.25	0.48	9.0	7.4	11.5	0 1	0 2	0 2	0-2 4	++	−	−	++
	5.0	31.25	0.48	10.5	12.0	13.8	0 1	0 1	0-2 3	0-3 3	++	−	−	+±
591	0	0	0.46	0.96	0.88	0.88	0 1	0 3	0-1 3	0 1	++	+++	−	−
	0.01	0.063	0.46	1.08	1.06	1.0	0 1	0 3	0 3	0 3	++	+++	−	−
	0.05	0.313	0.46	1.24	1.25	1.0	0 1	0 3	0 1	0 1	++	+++	−	−
	0.1	0.625	0.46	4.7	5.90	5.8	0 1	0 3	0-1 3	0 1	++	+++	−	−
	0.5	3.125	0.46	3.8	4.7	5.0	0 1	0 2	0-1 3	0 3	++	++++	−	−
	1.0	6.25	0.46	7.2	7.8	6.8	0 1	0 3	0-1 3	0 3	++	−	−	++
	5.0	31.25	0.46	8.4	11.5	10.5	0 1	0 2	0 1	0 4	++	−	−	++++



第1図 *M. glutamicus* に対する酵母エキス添加量の影響——生育および pH——
—— 菌体量 ---- pH

biotin 単独添加の場合より良好である．特に 516 株では2日目に最高 18 mg/ml の生育をしめした．582 株は（第1図）にも見られるように，biotin が比較的多量に存在する場合は2日を過ぎても緩慢な増殖を続けるが，酵母エキスの場合も同様で，0.1％以上 5％添加まで菌体量は経日的に増加する．pH の変動は生育と特にきんみつな関係を有することがしめされる．591 株においてもほぼ同様なことがいい得る．

酵母エキス添加の場合の菌形態も，biotin 添加の場合とまったく同じで，生育の不良な範囲では不完全分裂をおこし，不整形を呈し，従って多数の septa が認められるが，良好な生育をしめす程度に酵母エキスを添加した場合はほとんどが整一な類楕円形態を呈し，septa の存在も稀である．

4. biotin の代りに corn steep liquor を添加した場合の菌形態

biotin の代りに corn steep liquor を添加した．使用した corn steep liquor（以下 C. S. L. と略）は日本食品化工製のものであり，bioassay により biotin 含量は 75 mγ/g である．

C. S. L. 添加培地は，滅菌後いちじるしい沈澱を生ずるので，菌体量の測定はおこなわなかったが，biotin 単独添加に比して見掛け上はるかに良好な生育をしめした．これは含有される他のビタミン類，窒素源，その他の影響であろう．実験範囲の C.S.L. 添加培地の biotin 含量は 0〜7.5 γ/l という低濃度にもかかわらず，良好な生育をしめした．biotin 単独添加の場合良好な生育を示すためには 5 γ/l 以上添加しなければならないが，C.S.L. の場合は，0.5％，すなわち biotin 換算 0.2 γ/l 程度ですでに充分の生育をしめす．また，この程度の C.S.L. 添加ですでに菌形は不整形をしめすことなく，整一な類楕円形となり，septa の形成もほとんど認められない．このことは biotin が本菌の必須成分とはいえ，biotin に加うるに他の種々の栄養分を同時に添加した方が多細胞体を形成せずに良好な生育をおこない得ることをしめす．このことはすでに酵母エキス添加の場合にも知り得たことである．これらの観察結果を（第5表）にしめした．

しかしここで注意しなければならないのは，C.S.L. 添加の場合，培養3日においていずれもいちじるしい多細胞体を形成することである．これは C.S.L. 添加で初期生育が良好になるため，培地中に存在した微量の biotin が消費され，培養後期では完全分裂をおこない得なくなり，従って多細胞体を形成するのではないかと想像されるが，biotin の消長を測定していないのでこの点は単なる推論に止める．

第5表 *M. glutamicus* に対する corn steep liquor 添加量の影響——生育，pH 変化，septa および m. granule の形成——

Strain	CSL† %	γ/l Biotin††	pH 1 day	pH 2	pH 3	Septa initial		Septa 1 day		Septa 2		Septa 3		Metachromatic granule initial	Metachromatic granule 1 day	Metachromatic granule 2	Metachromatic granule 3
	0	0	6.2	5.2	5.4	0	1	1-3	5	2-3	4	1-3	5	+++	−	−	−
	0.1	0.075	5.0	4.8	4.8	0	1	1-3	4	1-3	3	1-2	3	+++	±	−	−
	0.5	0.375	5.0	5.0	7.4	0	1	0	1	0	1	1-2	3	+++	−	−	−
516	1.0	0.75	5.0	5.2	8.2	0	1	0	3	0	1	1-3	5	+++	−	−	−
	2.5	1.875	5.0	5.2	7.8	0	1	0	1	0	2	1-3	4	+++	−	++	−
	5.0	3.75	5.0	5.0	8.2	0	1	0	2	0	2	1-3	5	+++	−	+	+
	10.0	7.5	5.0	5.4	8.2	0	1	0	1	0	2	1-3	5	+++	−	+	±
	0	0	7.0	5.0	5.0	0	3	1-3	5	1-3	3	1-2	3	++	−	−	−
	0.1	0.075	5.0	4.6	4.8	0	3	1-3	4	1-3	5	1-3	4	++	−	−	−
	0.5	0.375	4.8	4.8	4.8	0	3	0-2	3	0	2	0-1	2	++	−	−	−
534	1.0	0.75	4.8	6.6	4.6	0	3	0-1	3	0	1	0-1	3	++	−	−	−
	2.5	1.875	5.0	4.8	7.6	0	3	0	1	0	2	1-3	4	++	−	−	++
	5.0	3.75	4.8	5.0	7.8	0	3	1	1	0	1	1-3	3	++	−	−	+++
	10.0	7.5	4.8	5.0	7.6	0	3	0	1	0	1	2-3	4	++	−	−	+++
	0	0	7.2	4.8	5.0	0	1	1-3	4	2-3	3	1-3	4	±	−	−	−
	0.1	0.075	5.0	4.8	4.6	0	1	2-3	5	2-3	4	1-3	4	±	−	−	−
	0.5	0.375	4.6	4.6	4.6	0	1	0	1	0-1	2	0-1	3	±	−	−	−
541	1.0	0.75	4.8	5.0	7.8	0	1	0	2	0	1	1-3	4	±	−	−	+++
	2.5	1.875	4.8	5.0	7.6	0	1	0	1	0	1	1-3	4	±	−	−	++++
	5.0	3.75	4.8	5.2	5.5	0	1	0	1	0	2	0	2	±	−	−	−
	10.0	7.5	4.8	5.0	5.4	0	1	0	1	0	1	1-2	4	±	−	−	++
	0	0	7.2	5.0	5.0	0	1	1-3	4	1-3	4	1-3	4	+++	++	−	−
	0.1	0.075	4.8	4.8	4.8	0	1	1-3	4	1-3	4	1	2	+++	−	−	−
	0.5	0.375	5.0	4.8	4.6	0	1	0	2	0	1	0	1	+++	−	−	−
560	1.0	0.75	4.8	5.0	7.4	0	1	0	2	0	1			+++	+++	−	+++
	2.5	1.875	4.8	4.8	7.8	0	1	0	1	0	1	2-3	7	+++	−	±	−
	5.0	3.75	4.6	4.8	5.0	0	1	0	1	0	1	0	1	+++	−	−	−
	10.0	7.5	4.8	4.6	4.4	0	1	0	1	0	2	0	1	+++	−	−	−
	0	0	7.4	4.8	4.8	0	3	1-3	5	2-3	5	1-2	4	+±	++	++	−
	0.1	0.075	4.6	4.4	4.6	0	3	1-3	4	1-2	3	1-2	3	+±	−	−	−
	0.5	0.375	5.0	5.4	7.6	0	3	0-1	3	0	3	1-3	4	+±	−	−	+++
582	1.0	0.75	5.0	5.2	8.2	0	3	0	0	0	0	2-4	6	+±	−	++	++
	2.5	1.875	4.8	5.4	8.2	0	3	0	1	0	3	1-3	4	+±	−	−	+++
	5.0	3.75	4.8	5.2	8.4	0	3	0	1	0	0	3-4	6	+±	−	−	++
	10.0	7.5	5.0	5.2	8.2	0	3	0	1	0	2	2-4	5	+±	−	−	++±
	0	0	4.8	4.6	4.6	0	1	1-3	5	1-3	6	1-3	4	+	−	−	−
	0.1	0.075	4.4	4.6	4.6	0	1	1-2	3	1	3	1-2	3	+	−	−	−
	0.5	0.375	4.8	5.0	7.2	0	1	0-1	3	0	1	1-2	3	+	−	−	+++
588	1.0	0.75	4.8	5.2	7.6	0	1	0-1	3	0	2	2-3	5	+	−	−	++
	2.5	1.875	5.2	5.0	7.8	0	1	0	1	0	1	3	6	+	−	−	+
	5.0	3.75	5.0	5.0	8.2	0	1	0	2	0	2	2-3	4	+	−	−	−
	10.0	7.5	5.2	5.4	8.2	0	1	0	1	0	1	1-3	4	+	−	−	−
	0	0	9.0	9.2	9.2	0	1	1-2	3	1-3	5	1-2	4	±	−	−	−
	0.1	0.075	6.0	4.6	4.6	0	1	1-3	4	1-2	3	1-2	3	±	−	−	−
	0.5	0.375	4.8	5.0	5.4	0	1	0	2	0	3	1	3	±	−	−	−
591	1.0	0.75	4.8	5.0	7.4	0	1	0	1	0	2	1	3	±	+	−	+++
	2.5	1.875	5.0	5.2	6.6	0	1	0	1	0	1	1	3	±	−	−	−
	5.0	3.75	4.8	5.6	7.0	0	1	0	1	0	1	1	2	±	−	−	+
	10.0	7.5	5.0	5.2	6.4	0	1	0	1	0-1	1	0	3	±	−	−	−

† corn steep liquor 添加量

†† bioassay による biotin 量

考 察

1. 菌形態について

M. glutamicus は，合成培地では培養の初期にいちじるしい形態変化をおこなう．すなわち，bouillon slant 上あるいは glucose bouillon 前培養では形は類楕円形を呈して整一であるが，これを合成培地（biotin 含量 $5\gamma/l$ 程度以下の場合）に添加すると数時間ないし10数時間の間に一見長肥大桿菌のごとく伸長する．この時期にはすでに数ケの septa が形成され，菌体は多細胞体を形成している．このような観察はすでに第1報においてのべたところであるが，biotin を $10\gamma/l$ 程度以上添加することにより，このような不整形菌体を形成することなく，完全分裂の結果整一な類楕円形菌体となり，しかも生育はきわめて良好となる．*M. glutamicus* に属する7株についての観察ではすべてこのような経過をしめす．

寒天培地においては，液体培地に比してこの不整形態の形成はいちじるしく（biotin $3\gamma/l$ 程度以下），この原因として考えられるのは寒天表層部の biotin のみ消費され，深部の biotin が拡散しきれず，biotin の欠乏状態を急速にまねくのではないかと想像される．液体培養の場合は，菌体が均一の懸濁状態で存在するため，biotin の消費も寒天上の場合ほど速やかではないと考えられる．

2. 生育について

合成培地に biotin を種々の濃度に添加した際の生育の有様を整理すると，ほぼ4型に分けられる．菌体量は optical density で測定したため，実際の菌数，菌体の大きさ，菌体内部成分の充実度などの要因が相互に影響するので，真に正しい菌の生育をしめすものではないが，このような型はかなり安定しており，それぞれの菌株の性質の一端をしめしているものであろう．

3. pH の変動について

biotin 量の多少，すなわち菌生育の度合に関連して pH は変化する．この変化を一括するとほぼ4型にわけられる．生育のようすはかなり強い再現性をもっているので，従って pH の変動も同程度の再現性をしめす．

4. m. grannle の形成

m. granule の形成は（第1表）にみるごとく，かなりの変動があるが，寒天培地の場合だと（第3表）にしめしたように規則的な形成をおこなう．菌株による形成のようすはほぼ4型にわけられるようである．

要 約

Micrococcus glutamicus に属する7株につき，菌形態と biotin 量との関係を観察した．

1. 生育について

合成培地に生育せしめるためには，培地に biotin を添加しなければならないが，その要求量は各菌株間に多少の差が認められる．

biotin を少量（$1\gamma/l$ 以下）添加した場合はいずれも生育は不良であるが $10\gamma/l$ 以上添加すれば良好な生育をしめす．

2. septa の形成について

本菌の異常肥大桿菌形態は，biotin の不足に起因する不完全分裂の結果おきた多細胞体の形成による．この場合多数の septa が形成され，見掛け上1個の菌体中に最高 11 個の septa が存在した場合もある．また biotin 濃度が同一であれば（ただし，biotin $3\gamma/l$ 程度以下）概して寒天培地上に生育した菌体の方が良く septa を形成する．biotin を $10\gamma/l$ 程度以上添加するとほとんどこのような septa の形成はおきない．

種々御指導を頂いた東大教授湯浅明博士，東大教授北原覚雄博士，東大助教授飯塚広博士に深く感謝する．また終始御鞭達下さった当所所長木下祝郎博士に感謝し実験に協力いただいた当所所員古川稔氏に謝意を表する．

文 献

1) 板垣史郎・木下祝郎，植雑 **72** : 51 (1959). 2) 板垣史郎，同 **73** : 258 (1960). 3) Mudd, S., Yoshida, A., and Koike, K., J. Bact. **75** : 224 (1958).

Summary

The effect of biotin-concentration on the growth and morphological character of *Micrococcus glutamicus* was studied, using seven strains of this organism.

1) Effect on the growth.

M. glutamicus requires biotin for its growth in the synthetic medium. However, some differences of the biotin requirement were observed among seven strains used.

Generally, at lower level of biotin-concentration (less than 1 γ/l), the cell growth of these strains was suppressed. On the other hand, at higher level of biotin-concentration (10 γ/l or more), good growth of cells was obtained.

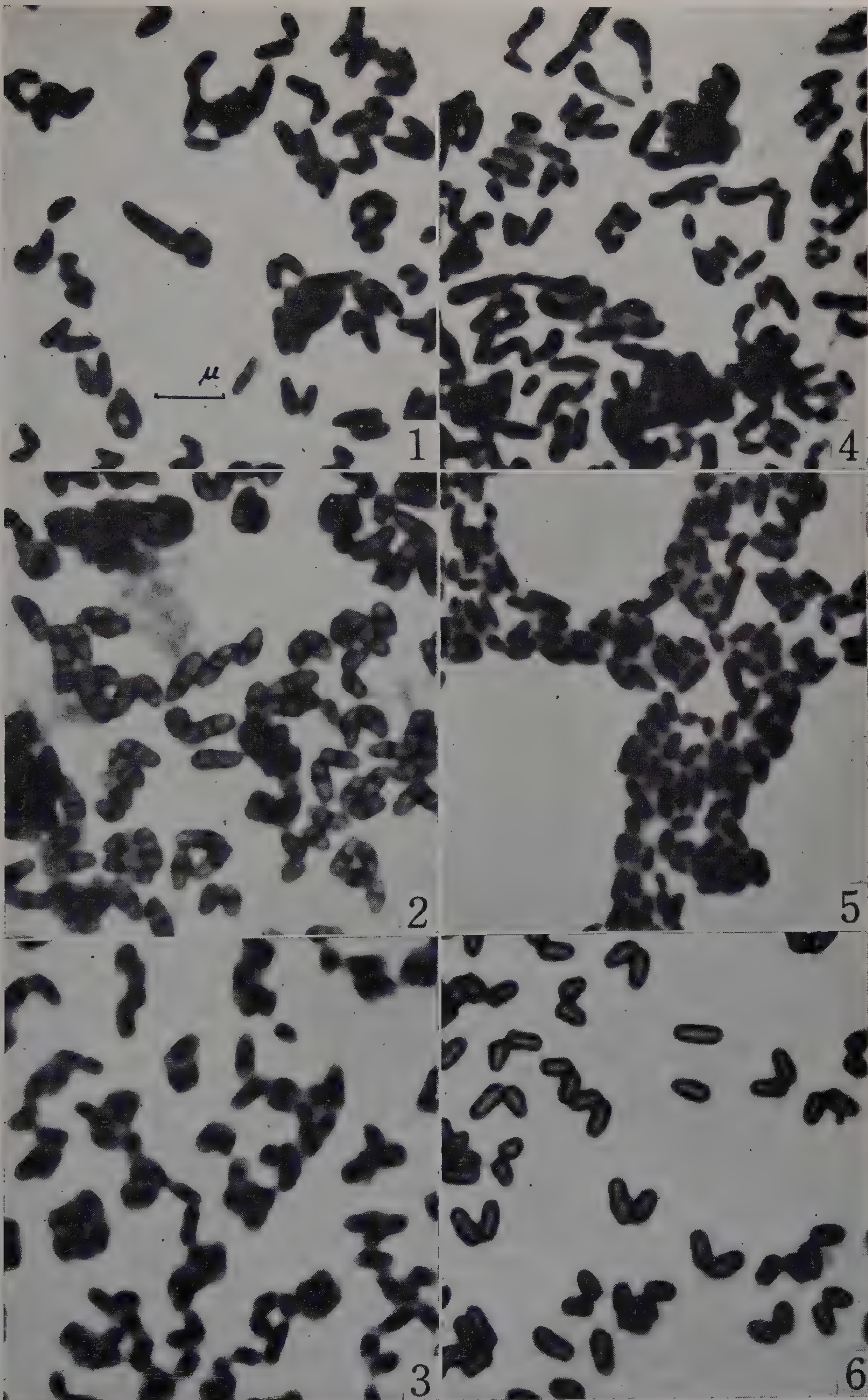
2) The effect of biotin-concentration on the formation of septa.

M. glutamicus usually formed elongated multicellular rod when it grew in the synthetic medium of biotin defficiency. In such case, many septa were found in the rod.

Under the same level of biotin-concentration (biotin 3 γ/l or less), the cells which grew on the slanted agar-medium formed more numerous septa than those in the synthetic liquid-medium. The formation of septa was not observed in most of the cases when those strains grew in the synthetic medium containing 10 γ/l or more biotin.

写 真 説 明

1. *M. glutamicus* 516, 合成培地 (biotin を含まず) 中にて 24 時間培養, methylene blue 染色.
2. 同上, 細胞膜染色.
3. *M. glutamicus* 588, 合成培地 (biotin 100 γ/l) 中にて 24 時間培養, methylene blue 染色.
4. *M. glutamicus* 541, 合成培地寒天斜面 (biotin を含まず) 上にて 24 時間培養. methylene blue 染色, いちじるしい不整伸長形を呈する.
5. *M. glutamicus* 534, 合成培地 (corn steep liquor 5 %含有) 中にて 3 日培養. 一たん整一な類楕円形態を呈した菌体が, この時期にふたたび伸長型を呈する。しかし, 菌はやや小型である. methylene blue 染色.
6. *M. glutamicus* 560, glucose bouillon 8 時間培養, 細胞膜染色, snapping division をおこなうと思われるようすがみられる.


μ
1
2
3
4
5
6

Bot. Mag. Tokyo **73**: 326–333 (July 25, 1960)

マツタケ菌の純粋分離と培養

広 本 一 由*

Kazuyoshi HIROMOTO*: Isolation and Pure Culture of the Mycelia of *Armillaria matsutake* S. ITO *et* IMAI, the Most Important Edible Mushroom in Japan.

昭和 35 年 1 月 7 日受理

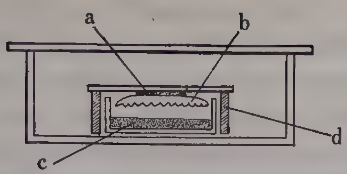
マツタケ菌の分離は従来，三村 (1915)[1]，増井 (1927)[2]，西門・山内 (1936)[3]，西門・木村・宮脇 (1941)[4]，浜田 (1950)[5,6]，藤岡・植原 (1957)[7])らが試みている．三村，増井らによると，マツタケ菌の純粋分離は容易にできるように思われるが，その後において同氏と同様な成果を得たという報告はないようである．西門・山内は土壌煎汁寒天，麦芽エキス寒天などの培養基を用いてマツタケ胞子から菌の分離を試み，その結果から「マツタケに限らず地上に発生しまたは菌根を形成する茸類胞子の発芽は普通の状態ではすこぶる困難で，これは欧米でも経験されている」と記載している．また，西門・木村・宮脇はマツタケ子実体から生長の迅速なマツタケ菌を分離したと報告しているが，それが真にマツタケ菌であるか否かについては疑問視されているようである．浜田はエビオス寒天培養基を用いて胞子および子実体の菌柄内部からマツタケ菌を純粋に分離したと報告している．藤岡・植原[7]) は浜田[5]) の方法を用いて菌柄，菌傘および胞子から 2 系統の菌，すなわち生育の遅いA群菌，生育のはなはだ速いB群菌を分離し，そのうち前者は浜田のマツタケ菌と称するものと同一であるとしているが，この菌をマツタケ菌と断定せずにマツタケ菌と仮定している．なお藤岡・植原の記載によると，浜田博士はB群菌は西門・木村・宮脇がマツタケ子実体から分離してマツタケ菌と断定したものと同一であるとし，このものを *Mortierella pusilla* と同定されたが筆者らは同定していないと述べている．すなわち，これら研究者間においては同一であるはずのマツタケ菌に対する見解がそれぞれ異なっている．要するにマツタケ菌の純粋分離はきわめて困難な問題として残されている状態である．著者は過去 10 年間マツタケ菌の分離を研究し，従来のものに比しきわめて良好な培養基を見出し，これを用いて胞子および子実体から分離した菌がマツタケ菌に間違いないという確証を得たのでここに報告する次第である．

本研究を行なうにあたり山口大教授日野巖博士，京大教授浜田稔博士，西条農短大教授田添元博士，同富永助教授，東大教授湯浅明博士，岡大木村劼二助教授，同武丸恒雄講師および水講助教授高井徹博士から懇切な御指導を戴いた．記して厚く謝意を表する．

* 山口県熊毛南高等学校, Kumage-Minami High School, Yamaguchi Prefecture, Japan.

実験の材料および方法

担子胞子から菌糸の純粋分離に関する実験の材料は山口県玖珂郡由宇町，同周東町，柳井市日積，同伊陸，同白潟の 5 か所の赤松林において，昭和 25 年から昭和 34 年まで春秋両季に発生したマツタケ子実体を採取して供試した．培養基は松葉煎汁寒天培養基を用いた．すなわちアカマツの青葉を短かく刻んだもの 50 g に井水または水道水 200 cc を加えて 1 昼夜放置したものを 10～20 分間煮沸した後放置してよく冷却し，その上澄を沪過して得た沪液に水を加えて 3～5 倍に希釈した液 100 cc に寒天 2 g を加えて煮てとかしたもので，その pH 値は 4.5～5.0 である．この培養基をあらかじめ乾熱殺菌をしておいたペトリー皿に流しこんで凝固させる．一方マツタケ子実体から菌傘の一部をなるべく培養基の全表面をおおうに足りる大きさに切り取り，これを清浄なガラス板にワセリンではりつけたものを第 1 図のように培養基の上にかかげる．このときガラス板はペトリー皿の縁に密着しないように僅かの間隙を保つ装置を施す．さらにこれを広いガラス槽に入れて蓋を施し，外部から塵芥などが入らないようにする．この装置を用いると，播種に長時間を要する



第1図　担子胞子の播種装置
a：ワセリン．b：菌傘．c：培地．
d：ガラス板がペトリー皿に密着しないための支台．

場合にペトリー皿内に水滴が生じたり，菌褶が湿って胞子の散布が阻害されるなどの心配がない．とくに梅雨期の湿潤なときにはこの装置を用いると好都合である．胞子の散布の難易は子実体の発育程度，新旧，湿度などによりいちじるしく異なる．胞子の播種時間は普通数分間で十分であるが，子実体の状態によりときには数時間または 10 数時間もかかることがある．また適当に成熟した子実体を用いてきわめて疎に播種する場合には1分間もかからぬことがある．播種が終ったらこれを定温器に入れて約 25° に保つ．

子実体から菌糸の分離に関する実験の材料は，昭和 32 年から昭和 34 年までの3年間春秋両季に発生したマツタケ子実体を用いた．材料採取地および培養基は上記のばあいと同じである．まずマツタケ子実体をかるく水洗して表面に附着している塵芥を除去し，沪紙で表面の水分を拭い去り，メスで菌柄の根元の中央に縦に 1～2 cm の切目を入れ，両手の指先で子実体を左右両半分に裂き割ると無菌的に新しい裂開面ができる．この裂開面に指頭を触れないように注意しながら，予め赤熱殺菌を行なっておいたメスで裂開面からマッチ箱形の小片を切取り，ペトリー皿の培養基表面に載せて蓋を施し 20～25° に保った．

実験結果

1. 担子胞子から菌糸の純粋分離

a）　胞子の発芽状態

胞子は早いものは 3～4 日で発芽するが，多くは 5～7 日頃から発芽が盛んになり2週間後には発芽すべき胞子のほとんど大部分が発芽を完了する．胞子は広楕円形または卵形で長径および短径はそれぞれ約 6.4μ, 5.6μ であるが発芽するときには一般に膨大して球形に近くなる．その時の径は $9～23\mu$ である．しかしほとんど膨大せずに発芽し，そのまま順調に生育するもの，また発芽後に膨大するものもある．しかし，胞子が膨大するだけで発芽しないものや膨大せずにそのまま発芽せずに終るものもある．発芽管は多くの場合1本出るが，2本出ることもある．そのとき2本の発芽管は互に直角，2直角およびその他任意の角度をなしている．

b）　発芽後の生育状態

胞子が発芽して間もなく枯死するものも少なくないが，多くは徐々に伸長分枝を続けて菌叢を形成するに至る．胞子が発芽した初めのうちは菌糸が比較的太く，その幅は 3μ 前後のものが多い．またところどころ膨れているものもあるが生育するにつれて細胞が漸次細長くなって，菌叢を形成する菌糸の細胞は幅が約 1.5μ, 長さが $60～100\mu$ あるいはそれ以上になる．培養基表面上において菌糸が伸びる速さは非常に遅いが，気中菌糸は比較的早く伸びるので，1か月後には培養基表面に小形粒状の白色菌叢が点々とできる．菌叢の拡がる速さは温度 23° において 10 日間に 1～2 mm 前後であるが気中菌糸の伸びの速さはおそらくその数倍に達すると思われる．ときには胞子が膨大して発芽し，伸び出た発芽管の先端がほぼ球形に膨大して，あたかも相隣る2個の胞子が同時に発芽し，向い合って発芽管を伸ばし双方の先端で接合したような形態をとっているものもある．また発芽管の先端に膨大部を生じ，そこから細い菌糸が伸び出し，その先端がまた膨大するものもあるが，このように膨大部分ができるものは，その生育が不良で多くは枯死する．

c）　発 芽 率

よく成熟していてバクテリアその他の微生物のいない新鮮で健全な子実体から得た胞子は 40% 以上発芽する．ときにはほとんど全部の胞子が発芽するが，10% 以下のことも少なくない．菌傘が上方にそり返った古いものや，7分開き，半開きなどの若いものから得た胞子でもかなり発芽する．発芽率および発芽後における菌糸の生育にいちじるしい妨害作用をおよぼすものの1つは菌傘に附着しているバクテリアその他の微生物である．しかし，同時につくった培養基を数箇のペトリー皿に入れてかため，同一菌傘部を用いて同一方法で播種し，同一温度に保っておいても，ペトリー皿が異なると発芽率にいちじるしい差を生ずることがある．しかもこの際バクテリアなどの微生物は全く発生しないのである．かかる事実はシメジ胞子の発芽試験の場合にもしば

しばみられるが，この原因については今後の研究で究明したい．

d） 胞子の播種密度と発芽後の生育

胞子の播種密度があまり大でない限り発芽率に顕著な差はみられないが，播種密度が非常に大になると発芽率は低下する．検鏡して胞子相互の間に間隙が認められる程度よりも疎であるときは，いくら疎であっても発芽率および発芽後の菌糸の生育はともに良好である．また非常に密な部分に発芽したものも菌叢にまで生育するが，この部分のものがとくによく生育するようなことはない．藤岡・植原[7]によると，胞子の播種密度がきわめて大きい部分に発芽したもの以外は生育しないとしているが，著者の培養基を用いると菌糸の生育に対して胞子の多数効果は認められない．

e） 春秋両季の材料で試験した結果の比較

春季および秋季産の材料を用いて胞子の発芽，発芽後における菌糸生育の速さおよび菌糸の形態などを比較した結果，両者間にいずれも差異は認められなかった．

f） 一核菌糸と二核菌糸

著者の培養基を用いると発芽率が大きく，菌糸も健全に生育するから一核菌糸の分離および分離した一核菌糸を接合させて二核菌糸を得ることもできる．また胞子をかなり密に播くと発芽後接合して二核菌糸になる．この場合一核菌糸と二核菌糸を外観的に区別することは不可能である．すなわちマツタケ菌の二核菌糸には普通に二核菌糸の特徴とされているところの嘴状突起がない．また菌糸の分枝の角度は一核菌糸でも二核菌糸でも，ともに直角に近いもの，それ以下のものなど種々であるから，シイタケやベッコウタケなどのように分枝角の大小で両菌糸を特徴づけることもできない．ただ1％酢酸カーミン液で核染色を行なって1細胞内にある核の数が1個であるか2個であるかを確めることによって両菌糸を区別することができる．

2. 子実体から菌糸の純粋分離

a） 菌糸の発生状態

移植後 1～2 昼夜を経過すると，移植片のところどころに菌糸が伸び出しているのが認められる．菌褶部からは菌糸の発生がとくに良好で，移植後約 12 時間で無数の菌糸が密に発生し始める．菌糸は，培養基表面に生じたものは無色であるが気中菌糸が集合したものは白色を呈する．菌糸の生育はきわめて遅いが，無数の気中菌糸が密集して一時に発生するから移植後 1～3 週間を経過した頃，移植片の周囲を注意深く観察すると肉眼でも白色の菌叢を認めることができる．このとき殺菌したピンセットで移植片を静かに取り去ると培養基表面に菌叢の一部が残る．この部分を別の培養基に移しておくと約1週間で白色の菌叢を形成する．この菌叢の一部分をとって1％酢酸カーミン液で染色すると1細胞内に2核を含んでいる．ピンセットで取った移植片を更に別の培養基に移しておくと，その周囲に間もなく新らしい菌叢が生じる．

b） 子実体の各部における菌糸の発生

発育が良好で健全な菌蕾の各部から移植片を切り取って培養した結果を第1表に示した．すなわち菌褶部からは最も早く無数の菌糸が密に発生し，1週間後には白色の菌叢が肉眼で認められる．菌柄部では移植当初には菌糸の発生が少ないが，しだいに多数の菌糸を生じ 3～4 週間後には白色の菌叢を肉眼で認めることができる．菌柄部からとった移植片において菌糸の発生数は菌柄の方向に平行な切口よりも直角な切口に比較的多いようである．健全な菌蕾の菌柄内部および菌褶においてはバクテリアその他の微生物が附着していない限りほとんど常に菌糸が発生する．とくに菌褶においてはそれが古くてもバクテリアが附着していない限りほとんど 100％の発生率を示す．質が比較的粗鬆な蓋肉および菌蕾において将来生長して蓋膜となる部位からは菌糸が発生しない．菌糸が発生しない移植片は移植後1か月を経過してもほとんど変色しないが，菌糸を発生する移植片は移植後間もなく変色して漸次褐色または黒褐色に変る．

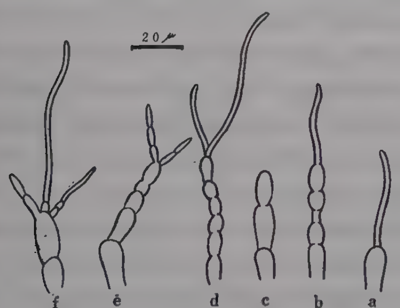
第1表 子実体の各部位における菌糸の発生

部位 ＼ 日数	1	2	3	7～21
柄 上 部	±	＋	⧺	白色の菌叢
柄 中 部	±	＋	⧺	同 上
柄 下 部	±	＋	⧺	同 上
蓋 肉	−	−	−	−
菌蕾において生長してベールになる部位	−	−	−	−
菌 褶	⧺	⧻	⧻⧻	白色の菌叢

＋：新しく菌糸が伸び出たことを示す．＋の多少は菌糸発生の良否の傾向を示す．－：菌糸が発生しないことを示す．±：菌糸の発生がまだ確認し難いことを示す．

c） 移植片から生ずる菌糸の生育状態

菌褶の一部を培養基表面に移し，それから発生する菌糸の生育の状態を観察すると，子実層における側糸の先端から菌糸が新たに伸び出るのをはっきりと認めることができる．ここに新らしく伸び出る菌糸は側糸の太さに比しいちじるしく繊細である．ときには側糸の先端にきわめて短い細胞が数箇できて，その最先端の細胞から繊細な菌糸が 1〜3 本伸び出て生育を続ける場合もある．子実体から発生した菌糸の生育の速さは，胞子から生じた菌糸と同様にきわめて徐々であるが，移植片を培養基に移すと間もなく多数の菌糸を発生するので，胞子発芽によるよりもはるかに早く菌糸を分離することができるとくに菌褶部を用いると分離がきわめて容易である．したがって二核菌糸の分離には子実体の菌褶部を用いるのが最も適当である．菌柄内部を移植した場合には，ときに移植片から発生した菌糸の先端に球形の膨大部を生じて，そのまま生育が終るもの，またはその膨大部から細い菌糸が伸び出しその先端にまた球形の膨大部を生じて伸長するものもある．あるいはまた，比較的小形の膨大部が数箇またはそれ以上珠数状に連なり，その先端の球形部から細い菌糸が伸び出るものもある．このような生育をするばあいには，菌糸は比較的太くて細胞の幅は普通の培養菌糸の幅の 2〜5 倍もあるが細胞の長さは比較的短い．したがって形態的には全く別種の菌のように思われることもあるが，核染色を行なうとやはり1細胞に2核を有するから，これはマツタケ菌糸が異常な生育をしたものと思われる．異常な生育をする菌糸は普通の菌糸よりもその生育が劣っている．



第2図 子実層から菌糸の発生

a： 側糸先端から繊細な菌糸が1本発生したもの．b： 側糸の先端に数箇の球状の細胞を生じ，その先端から繊細な菌糸が1本発生したもの．c： 側糸の先端に短かい細胞が2個できたもので繊細な菌糸はまだ伸び出ていない．d，e および f： 繊細な菌糸が 2〜3 本発生したもの．

d） 子実体の状態と菌糸の発生

マツタケ子実体の初期の菌蕾，菌傘が上方にそり返り菌褶が褐色を帯びた老子実体，採取直後の新鮮な子実体および採取後相当の日数を経過した古い子実体などの各材料から菌褶および菌柄内部を切取って培養した結果は古いものでは菌柄部からの菌糸の発生はきわめて悪いが，菌褶部においては菌糸が密に発生し老幼新古の間にいちじるしい差異は認められない．ただあまりに老いた子実体や採取後長く保存して古くなったものでは，バクテリアその他の微生物が多くなるので好結果が得られない場合が多い．しかし，採取直後の材料でも好結果が得られないことがあるが，これは多くはバクテリアその他の微生物が発生しているためである．

e） 子実体から分離した菌糸と子実体を構成する菌糸との比較

子実体から分離した二核菌糸と胞子から導いた二核菌糸とは形態や生育の状態などがよく一致しているので両者の区別はできないが，子実体を構成する菌糸の細胞は子実体から分離した菌糸の細胞よりもよく膨れていて非常に大きく細胞の長さは培養菌糸のそれよりもはるかに短い．培養菌糸の細胞の長さは 60〜100 μ，巾は 1.5 μ 前後であるが，子実体を構成する細胞の長さは培養菌糸の細胞の長さの 1/2〜1/5，巾は 5〜8 倍である．培養基の中で異常な生育を示す菌糸の巾は普通の培養菌糸の巾の 2〜5 倍ぐらいである．細胞内における2核は細胞のほぼ中央に位置する．2核間の距離は子実体の細胞では多くは 4 μ 前後であるが，培養菌糸では普通 18 μ 前後のものが多く稀には 4 μ 前後のものもある．子実体の細胞には1細胞内に相接近して4核，8核，10核など多数の核を有することが珍しくないが，培養菌糸においては多核の細胞は見当らない．

3. 培養菌糸とマツタケの香気

担子胞子または子実体から分離したマツタケ菌にはマツタケ特有の香気がない．その原因は香気を生ずるために必要な成分が培養基中に欠けているためであると思われる．著者はマツタケ発生地の土壌からマツタケ菌を分離しようと試みている間に，マツタケ菌よりも非常に生育の速い（10 日間に 14 mm

前後伸びる）1種の菌* を分離したが，この菌を松葉煎汁寒天基上で培養すると自然発生のマツタケ菌とは全く異なった臭を発するが，この菌を2％の蔗糖を加えた松葉煎汁寒天基上に移すと 2〜3 週間後にはマツタケ菌に類似した香気が発生し，またこの培養基にさらに馬鈴薯およびエビオスの煎汁を加えると香気が一層強くなる．この事実から推して，培養マツタケ菌がマツタケ特有の香気を発生しないのは，培養基中に菌が香気を生産するために必要な成分を欠くことに起因すると思われる．

4. 菌糸の生育に影響する諸条件

a） 培養基の濃度

松葉煎汁液を水で2〜10倍に希釈して種々の濃度の寒天培養基をつくって菌糸を培養してみると，2倍基（2倍に希釈した培養基）では気中菌糸も培養基表面の菌糸も生育が非常に悪い．3〜4 倍基では気中菌糸の発生が密でその伸びも速いが培養基表面上における菌糸の伸びは遅い．5〜6 倍基では気中菌糸が少なくなるが培養基表面上における菌糸の生育は比較的速い．7〜10 倍基では気中菌糸はさらに少なくなるが培養基表面上での菌糸の伸びは比較的速く 5〜6 倍基程度である．以上の結果から濃度が小となるにつれて培養基表面上に拡がる菌叢は疎になる傾向が認められる．

b） 松葉煎汁寒天基に他の成分の添加

松葉煎汁寒天基に葡萄糖（2％），馬鈴薯煎汁，エビオス煎汁 (0.1〜0.5％)，ビオチン[8] (1 l 中 0.1〜100 γ)，土壌煎汁などを別々に，またはこれらのうちの幾つかを同時に添加しても菌糸の生育にいちじるしい影響はない．

c） 水素イオン濃度

松葉煎汁寒天培養基の pH 値を種々に変えてマツタケ菌を培養した結果，最も良好な生育を示した pH 値の範囲は 4.0〜6.0 であった．

d） 温　度

松葉煎汁培養基の温度を 5° から 33° の範囲において 10 区分し，それぞれの菌糸の生育状態を観察した結果，生育の最も良好であった好適温度は 23〜26° であった．

考　　察

従来培養基上でのマツタケ胞子の発芽がきわめて困難なことは多くの研究者が等しく認めているところである．したがって多数のマツタケ胞子の中からきわめて稀に発芽して生じた菌を直ちにマツタケ菌と断定し難くなるわけである．西門・山内[3]は多数のマツタケ胞子の中から嘴状突起のある双核菌糸を分離し，これをマツタケ菌と同定したが，同時にマツタケ胞子は発芽が困難なことおよび単個簡養には成功しなかったと述べている．発芽率のきわめて低い多数の胞子のなかから嘴状突起のある菌糸が稀に生じたとしても，単個培養にも成功せずしてそれを直ちにマツタケの二核菌糸と断定することには疑点があるように思われる．著者がマツタケ胞子の単個培養を行ない，これらの間で交配を行なって得た菌糸を酢酸カーミン液で染色して二核菌糸であることを確かめた菌糸には嘴状突起は全く認められない．また子実体を構成している菌糸および子実体から分離した菌糸にも嘴状突起は全然見当らない．富永[9]はマツタケの二次菌糸が細胞分裂を行なうときには嘴状突起を形成するが，単に細胞膜の一部がふくれるのみで所謂突起と称するほどでない場合もあると報告している．けれども著者は二核菌糸の最先端の細胞を検鏡しているうちに1細胞内の2核がそれぞれ分裂して4核になっているものを観察した．その場合に細胞膜に嘴状突起の用をなすと思われるような特殊なふくらみは決して認められなかった．以上の事実から2核菌糸の細胞分裂に際しては，嘴状突起は勿論，細胞膜に僅かなふくらみも分裂のために生ずることはないと見るのが至当のようである（なお Sass[10]参照）．藤岡・植原[7]は浜田[5,6]の方法を用いて胞子の発芽実験を行ない，その発芽率はきわめて僅少で 0.01％ 以下であると推定している．なおまた，播種した胞子間に空隙が認められない程度以上に密でないと発芽した胞子が生育しないとし，胞子発芽の後に菌糸が生育するためには胞子密度が大なることが必須条件であるとしている．したがって1個の胞子が発芽して菌糸が生育する状態を観察していない，このことは発芽率のきわめて低いことと合わせ考えると，そこに生じた菌叢を確実にマツタケ菌だと断言し難いように思われる．さらに藤岡・植原[7]は子実体からも胞子から分離したと同じ菌を分離し，その菌は浜田[6]がマツタケ菌と呼ぶものと同一であるとしているがこの菌をマツタケ菌と断定することを避けてマツタケ菌と仮定している．すなわち浜田と藤岡・植原との間には同一方法で生じた同

* この菌の学名は不明であるから本文では仮りにH菌と呼ぶこととする．

一の菌に対して幾分意見の相違がある．従来の研究では1箇の胞子から出発して菌叢を形成するまでの過程を観察した報告も，子実体から分離した菌糸が間違いなくマツタケ菌糸であるという確証を示した報告もない．著者の培養基を用いると発芽率がきわめて大で，かつ播種密度は如何に疎であってもよく発芽して生育するから，1個の胞子が発芽して菌叢を生ずるまでの過程を詳しく観察することができた．したがって著者が胞子から分離した菌はマツタケ菌に間違いないことが明らかである．著者はさらに著者の培養基を用いて菌褶部からきわめて容易に菌の分離ができることを見出し，その際子実層に無数に存在する菌糸の最先端たる側糸から新らしく菌糸が伸び出る状態を検鏡することができたし，そしてそこに生じた菌糸に核染色を行なってこれが2核菌糸であることおよびこの菌糸は胞子から導いた2核菌糸と同一であることを確めることができた．だから子実体から分離した菌糸をマツタケ菌糸と断言しても間違いないと思う．なお，子実体から菌を分離するにあたり，従来多くは菌柄内部を用いているが，マツタケに限らずシイタケ・シメジ・マツタケモドキ[11−13]その他多くの菌蕈類子実体について実験した結果も，菌糸が最もよく伸び出る部位は子実層である．この理由はおそらく子実層に多数存在する菌糸の最先端が最も生活力に富むため，これが適当な培養基に移植されると直ちに生長を開始するからであろう．子実体が古くなって菌柄部からは決して菌糸を発生しない場合でも，菌褶部を用いると好結果を得る場合がきわめて多いのもこのためであると思われる．著者は菌糸のこのような分離法を仮りに子実層分離法と呼び，これに関しては別報で詳細な報告をするつもりである．胞子および子実体から分離したマツタケ菌にはマツタケ菌特有の香気がないが，その理由については培養基中に香気を生産するに必要な要素の欠如に起因することを，H菌を一例にとって実験的に証明することができた．したがって培養菌糸にマツタケ菌特有の香気がないことをもって，これをマツタケ菌でないとする議論は当を得ていない．胞子から菌糸を分離するに際し，ときに菌糸のところどころに球形の膨大部を生じ，生育が普通の菌糸よりもさらに遅く，あたかも別種の菌のように思われるものを生ずることがあるが，この原因はおそらく胞子の成熟不十分のためか，あるいは成熟は十分であったとしても発芽前に何等か不適当な環境におかれたために胞子の生活力が衰えたことによると思われる．このことはシメジ胞子を長期間貯蔵してその発芽力が低下した場合にも上記と類似の現象が起こることから推定することができる．すなわち著者はシメジ胞子を 11 月末に採取して翌春3月末まで室温にて貯蔵し，1週間ごとに発芽試験を行なったが，この実験中，2月になると発芽開始が遅くなり，発芽率もいちじるしく低下し，発芽してもそこに生じた菌糸が不規則にふくれて生育がいちじるしく遅くなり，ついには枯死するものも多くなった．あるいはまた，菌糸の先端に小さい球状部ができており，その菌糸がきわめて徐々に小刻みの波形に伸びるなど全くシメジ菌糸とは思われない形態をとるものができたが，枯死しないものは 3～4 週間後には再び正常の菌糸を伸ばして普通の速さで盛んに生育して他の1核菌糸と接合して2核菌糸になるものもあった．これは長期間の貯蔵により胞子の生活力が衰え，かろうじて発芽してそのまま枯死するもの，あるいは再び生活力を回復するものなどがあるためと思われる．マツタケ菌糸が異常形態をとるのもシメジ菌糸の場合と類似現象と思われる．また菌柄内部から菌糸を分離する場合にも，菌糸に多数の球形の膨大部を生ずることが多いが，これもおそらく菌柄部菌糸の生活力の減退が主因であると思われる．

摘　　要

1. マツタケ胞子は松葉煎汁寒天培養基の表面においてよく発芽し，その後 2～3 週間で白色の菌叢を生ずる．培養基の pH 値は 4.5～5.0 である．

2. 担子胞子は温度約 25° において 4～15 日間で発芽すべき胞子の大部分が発芽を完了する．

3. 担子胞子の発芽率は良好な場合には 40% 以上に達する．

4. 松葉煎汁寒天培養基を用いると子実体から容易に菌糸を分離することができる．

5. 子実体から菌糸を分離するのに最適の部位は菌褶部である．菌柄内部からも分離できるが菌褶部には劣る．蓋肉および菌蕾において将来生長して蓋膜となる部位は菌糸の分離に不適当である．

6. 健全な材料から得た菌褶部を用いると，分離の成功率はほとんど 100%である．

7. 分離した菌糸は胞子発芽用培養基上でよく生育する．

8. 菌糸は通常 8〜30° で生育し，最適温度は 23〜26° である．

9. 培養基表面上における菌糸生育の速さは 10 日間に約 1〜2 mm であるが気中菌糸の伸長の速さはおそらくその数倍と思われる．

10. 単一胞子から得た一核菌糸と，それらを混植培養して得た 2 核菌糸とは外観上区別できない．両菌糸は 1 %酢酸カーミン液で核染色を行なうことにより区別される．

11. 子実体から分離した菌糸と胞子から導いた 2 核菌糸とは，その性質がきわめてよく似ているので両者を区別することはできない．

12. 子実体から分離した菌糸は，子実体を構成する菌糸よりもいちじるしく細くて長い．

13. マツタケ発生地の土壌から分離した生育迅速なある種の菌（H菌と仮称）に対する培養基の組成を変えると，マツタケに類似した香気を発生する場合と，全く異なった臭を発する場合とがある．この 1 例からマツタケの培養菌糸がマツタケ特有の香気を発生しないのは香気を生産するに必要な要素が培養基中に欠けていることに起因すると思われる．

文　　献

1) 三村鐘三郎，林業試験場報告，**7** : 93 (1914).　2) Masui, K., Coll. Sci. Kyoto Imp. Univ., Mem. **3** : 149 (1927).　3) 西門義一・山内己酉，大原農研報告，**7** : 273 (1936).　4) 西門義一・木村劼二・宮脇雪夫，大原農研報告，**8** : 433 (1941).　5) 浜田　稔，植雑，**63** : 40 (1950).　6) 浜田　稔，自然，**8** : 56 (1953).　7) 藤岡保夫・植原一雄，日本菌学会報，No. **6** : 10 (1957).　8) 植原一雄・藤岡保夫，広島農業短大研究報告，**1** : 6 (1958).　9) 富永保人，広島農業短大研究報告，**1** : 1 (1958).　10) Sass, J. E., Amer. Jour. Bot. **16** : 663 (1929).　11) 川村清一，原色日本菌類図鑑 (1953).　12) 今関六也，原色日本菌類図譜 (1957).　13)朝比奈泰彦，日本隠花植物図鑑 (1924).

Summary

(1) The spore of *Armillaria matsutake* S. Ito *et* Imai germinates well on such a culture medium as pine-needle extract agar, pine-needle decoction agar, or rotten-leaves decoction agar, and in two or three weeks after the germination, the mycelium is found white in color.

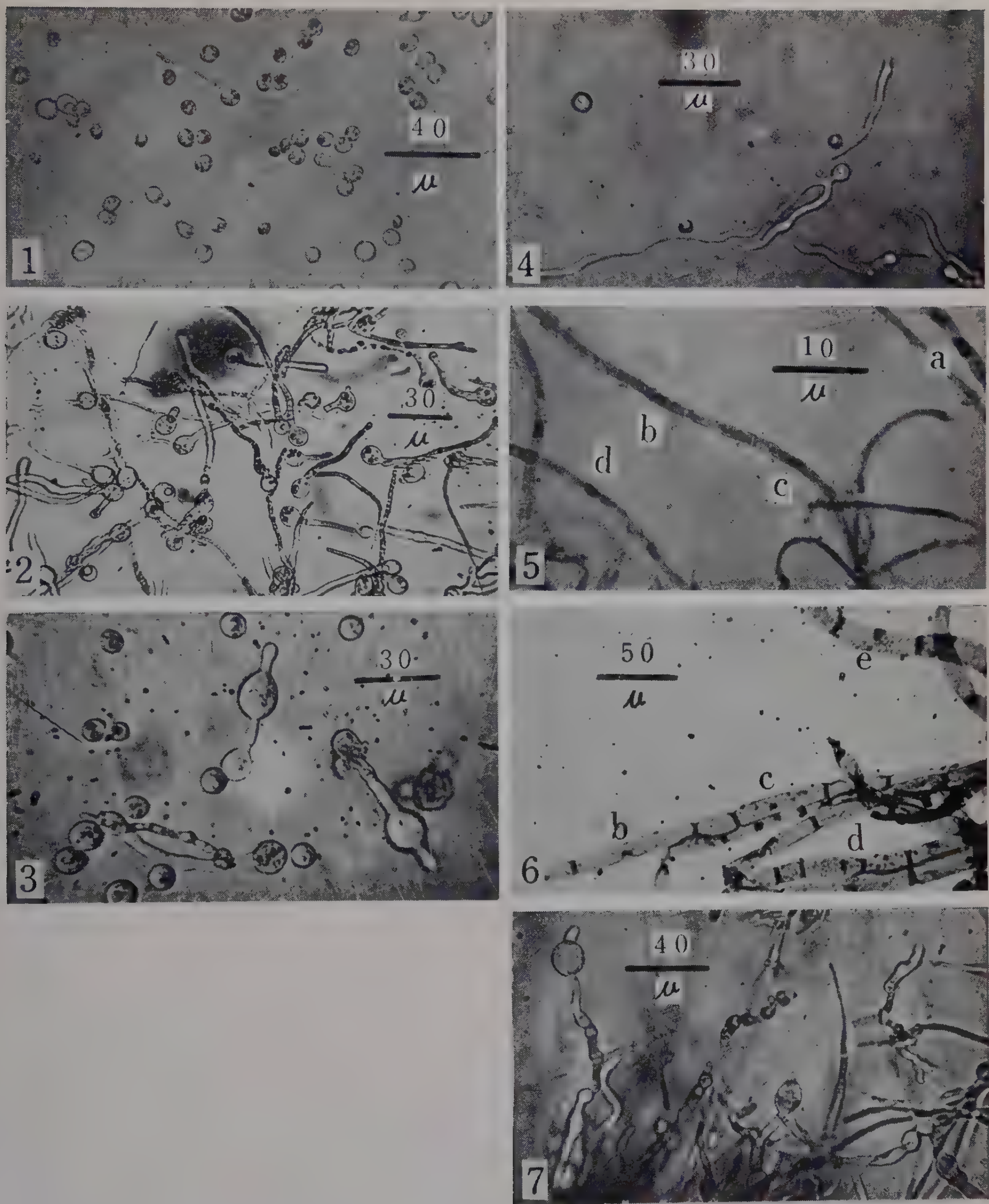
(2) When such a culture medium as pine-needle decoction agar or pine-needle extract agar is used, the mycelium can easily be isolated from the fruit body.

(3) When we make use of the gill part taken from a sound material, the isolation of the mycelium is almost always successful.

(4) No apparent difference is observed between the mono-nucleate hyphae originated from a single spore and the bi-nucleate hyphae created by mixing many spores. The former is, however, easily distinguishable from the latter by staining the nuclei with 1% aceto-carmine.

(5) The hyphae isolated from the fruit body and the bi-nucleate hyphae created by mixing many spores are very much alike in the natures shown by them, and are difficult to distinguish from each other.

(6) A fungus which was isolated from the soil and grew rapidly on the culture medium (provisionally called H-fungus) emits a scent very much like that of *Armillaria matsutake* when a special medium containing a certain ingredient is used, and in another case, it emits a rather unpleasant scent. From this fact, it seems to be possible to conclude that the hyphae of *Armillaria matsutake* do not emit a fragrant scent on the culture medium, because of the lack of a certain element necessary for the hyphae to produce the fragrance.



第 1 図 版

1) 胞子発芽 (5 日). 2) 同 (10 日). 3) 同，膨大いちじるしきもの (10 日). 4) 発芽後生育良好なもの (14 日). 5) 子実体から分離した繊細な 2 核菌糸， a： 2 核が相接近している， b および c： 2 核間距離が 17.5 μ， d および e： 2 核間距離が 10 μ. 6) ベールの菌糸， a： 2 核間距離が 8 μ， b， c および d では 2 核がいちじるしく接近している， e： 1 細胞内に 4 核を含む. 7) 菌柄部から生じた異常菌糸で膨大部が多い.

Bot. Mag. Tokyo **73**: 334–335 (July 25, 1960)

Short Communication

Yukiyoshi Ogawa*: Über die Auslösung der Blütenbildung von *Pharbitis Nil* durch niedere Temperatur

小川幸持*: 低温によるアサガオの花芽形成

Eingegangen am 15, Juni 1960

In ihrer Arbeit über den Einfluss der Temperatur auf Photoperiodismus verschiedener Pflanzen haben Roberts und Struckmeyer[1]) (1939) berichtet, dass *Nicotiana tabacum*, Rasse Maryland Mammoth, eine typische Kurztagpflanze, zur Blütenbildung unter Langtag kommt, falls die Pflanze bei niederer Temperatur gezogen worden war. Darrow[2]) (1936) fand ein ähnliches Phänomen bei *Fragaria*, die nach ihm eine Kurztagpflanze sein soll. Oft beobachten wir auch bei *Pharbitis Nil*, Rasse Kidachi, Pflanzen mit Blütenknospen unter Dauerlicht bei absteigender Temperatur im Herbst. Um den Einfluss der Temperatur auf das photoperiodische Verhalten dieser Pflanzen näher zu erforschen, wurden einige Untersuchungen ausgeführt.

Die Pflanzen wurden auf bereits beschriebene Weise im Gewächshaus bei 30° gezogen[3]). Vor dem Versuche wurden 30 bis 40 Tage alte Pflanzen 2 Tage lang bei 20° gelagert und dann wurde eine Gruppe von Pflanzen der Thermoperiode ausgesetzt, die aus der 16 stündigen Kalteperiode von 10° und der 8 stündigen Warmeperiode von 20° besteht. Andere Pflanzen wurden dauernd im Zimmer bei 10° belassen. Kontrollpflanzen erfuhren keine Kältebehandlung. Nach 5, 10 und 14 Tagen wurden die Versuchspflanzen auf 30° zurückgebracht und dort bis zur Ernte gezogen.

Tabelle 1. Blütenbildung von *Pharbitis Nil*, Rasse Kidachi, unter Dauerlicht* durch Kältebehandlung.

Versuchsnummer	Temperatur	Reaktion	Dauer der Kältebehandlung in Tagen			
			0	5	10	14
I	10°	Blühverhältnis	0/16	4/17	14/17	11/13
		Blühprozent	0	23	82	84
		Blütenzahl pro 10 Pflanzen	0	3.5±1.5	10.6±1.6	20.7±3.7
II	Thermoperiode, die aus der 16 st. Kälteperiode von 10° und der 8 st. Wärmeperiode von 20° besteht.	Blühverhältnis	0/21	5/10	16/16	12/12
		Blühprozent	0	50	100	100
		Blütenzahl pro 10 Pflanzen	0	9.2±2.7	21.2±1.8	20.0±2.1

* Dauerlicht von ca. 3000 Lux.

Wie man aus Tabelle 1 ersehen kann, rief schon 5 tägige Kälte bzw. thermoperiodische Behandlung eine ausgeprägtere Blühförderung hervor. Ähnliche, aber etwas schwächere, Reaktionen wurden auch bei Rasse Violett beobachtet.

* Laboratorium der Angewandten Botanik der Landwirtschaftlichen Fakultät, Kyoto Universität, Kyoto, Japan. 京都大学農学部応用植物学教室.

Tabelle 2. Thermoperiodische Blütenbildung von *Pharbitis Nil*, Rasse Kidachi, in Abhängigkeit mit der Entblätterung.

Reaktion	Thermoperiodische Behandlung*		Kontrolle ohne Kältebehandlung
	Blätter intakt	Blätter beraubt	
Blühverhältnis	13/13	4/17	0/20
Blühprozent	100	23	0
Blütenzahl pro 10 Pflanzen	16.1±1.4	2.3±1.0	0

* 10 Tage von 16 stündige Kälteperiode von 10° und 8 stündige Wärmeperiode von 20°.

Um die Rolle von Blättern bei der Blütenbildung durch Kälte zu prüfen, wurden eine Reihe Pflanzen aller Blätter beraubt und wie oben behandelt (Tabelle 2). Eine starke Blütenbildung war nur bei den intakten Pflanzen zu beobachten. Ähnliche Ergebnisse wurden auch bei der Rasse Violett bestätigt.

Tashima und Imamura[4]) (1953) haben angegeben, dass bei einer Temperatur schwankend von 10° bis 15° im vollständigen Dunkel sterilgezogene Keimlinge von *Pharbitis* zur Blütenbildung veranlasst werden konnten, wobei nach ihnen der Lichtabschluss am bedeutsamsten war. Es scheint jedoch nicht ausgeschlossen zu sein, dass die dabei benutzte, niedere Temperatur für die Ausbildung der Blütenanlagen verantwortlich ist. Neuerdings ist eine günstige Wirkung von niederer Temperatur auf die Blütenbildung unter Kurztag bei *Xanthium* von Zeeuw[5]) (1957) und bei *Bryophyllum* von Schwabe[6]) (1957) beobachtet worden.

Literaturverzeichnis

1) Robert, R. H., and Struckmeyer, B. E., Jour. Agr. Res., **56**: 633 (1939). 2) Darrow, G, M., Proc. Amer. Soc. Hort. Sci. **34**: 360 (1936). 3) Imamura, S., Proc. Jap. Acad. **29**: 268 (1953). 4) Tashima, Y., and Imamura, S., Proc. Jap. Acad. **29**: 581 (1953). 5) Zeeuw, de D., Nature, **180**: 558 (1957). 6) Schwabe, W. W., International Union Biological Science. B: 95 (1957).

本　会　記　事

支部通信

(北海道支部)

二月例会（2月27日，北大・農において）

佐々木喜美子: *Azotobacter vinelandii* におけるコハク酸化酵素系の生成について．村山大記: 欧米めぐり．

(関東支部)

六月例会（6月18日，東大・理・植において）

竹内正幸: 北部アマゾンの植相

(中部支部)

第8回支部大会（5月29日，名大・理において）

南川　洋: 藤原岳およびその周辺の植物群落．井波一雄: 愛知県の植物分布地理．高木典雄: 表日本における蘚類分布の一断面．特に尾張，美濃地方について．牛山六男: 私のみた縞枯現象と偏心生長について．神谷　平: 接合藻類細胞の電子顕微鏡観察．杉浦昌弘: 発芽種子の呼吸について．藤井良平: ウキクサの越冬芽の形成．土井田幸郎: イネ属植物の発生学的研究．I．イネ属数種の胚嚢形成および発生学的にみた半不稔イネの不稔原因．川松重信: 定位澱粉粒説について．須賀瑛文：長野県の輪藻類 (Charophyta) について．脇田晴美: 伊勢湾台風の植物におよぼした影響．とくに海水の長期浸入地域における水生植物の被害．丸山槇子・岩塚　寿: いおう細菌の化学合成の機作．市村国彦・井沢三生・太田行人: 発芽種子の可溶性 RNA．森　隆也: 花粉の予措について．熊沢正夫: ヒガンバナ科鱗茎の形態学再報．

(近畿支部)

支部大会（4月24日，京大・理・植において）

高木虎雄: ササ・シノ・ネザサ属の小花の形態．梅崎　勇: 日本産ベニモズク属二種の生殖器官の発達について．藤田安二: コウヨウザンとランダイスギ．上野実朗: イチョウ，ソテツ，マオウの花粉膜の微細構造について，

(九州支部)

第59回例会（4月23日，九大・教養において）

宮田逸夫: 着生こけ類の光合成速度およびクロロフィル含量の季節的変化．千葉保胤: 葉緑体の微細構造について．

第10回支部大会（5月28日，29日，九大理・農において）

中山孝則: 万年山の森林植生．鈴木時夫・町田瑞生: 5万分の1大分図幅に図示されたる植物社会について．鈴木時夫: 九州中部山地のスダシイ群団．鹿子木憲章: 刈り取り量曲線にあらわれたススキ群団の生態学的特徴．鴨川　誠・秋武和俊: ヤドリギ類の寄主選択について，(I)宿主樹上の分布について．秋武和俊・鴨川　誠: ヤドリギ類の寄主選択について，(II)種子発芽について．吉田　稔: 分解速度からみた落葉層の組成，特に植物群落の特性．楠　元司: 常緑広葉樹の低温抵抗性について．秋山文司: 桑園内の光条件について．新　敏夫: 日本産ツヤゴケ属蘚類について．野口　彰: 蘚類の胞子発芽の形成．森　通保: 茶色カワモズクの輪生枝叢の形態について．中村和郎: アカパンカビにおける交叉と温度との関係．江頭　威: アカパンカビの受精における分生子間の競争．井上　覚: アレチノギクとオオコウギク雑種に関する細胞遺伝学的研究．千葉保胤・佐藤孝彦: 葉緑体中の RNA の塩基組成．菅原　淳・千葉保胤: 葉緑体への P^{32} のとりこみ，(1) 葉緑体における P^{32} の分布．東　四郎: 根粒中の B_{12}．山根銀五郎・奥　達夫: キノコの B_{12}．この支部大会は，動物学会，生態学会との合同大会として開催された．

Bot. Mag. Tokyo, **37**: 337—340 (September 25, 1960)

Cytological Studies in *Polygonum* and Related Genera I.*

by Yukio DOIDA**

Received December 21, 1959

Polygonum and its related genera have been submitted to cytological studies by many investigators[1-8]. But the karyotypes of the members of the genus and its relatives have been scarcely studied in detail, as their chromosomes are so small that a karyotype analysis is difficult. They are also hard to stain. Darlington and Janaki Ammal[9] and Darlington and Wylie[10] reported various basic chromosome number for Polygonaceae, for instance b=8 for *Fagopyrum****, b=10 for *Muehlenbeckia*, and b=10 and 11 for genus *Polygonum*. Löve and Löve investigated species of genus *Polygonum* found in eastern North America from the cytotaxonomic point of view[7]. They reported that the basic number may be 10 and the chromosomes are mostly V-shaped. The species studied by them range from diploid (2n=20) to octoploid (2n=80).

The present author is studying *Polygonum* and its relatives from the cytological and embryological view points[11-13]. The results of his cytological investigations of species mostly native in Japan are reported in this paper.

Materials and Methods

The large majority of the materials were collected by the author at several localities in Japan, mostly in Misima and the neighbouring areas. Some of the materials were supplied by Koishikawa Botanic Garden in Tokyo, and Higashiyama Botanic Garden in Nagoya. Table 1 lists the species, the chromosome numbers and the localities where the materials were obtained.

Root tip cells and pollen mother cells were used for studies. The anthers were fixed with Farmer's fluid for a little longer than one hour and 1 per cent aceto-carmine was used for staining of meiotic chromosomes. The following method was adopted for making temporary preparations of chromosomes of root tip cells: About 1 cm. long root tips were treated with 8-hydroxyquinoline (0.002 M) at 4°-20° for 4 hours. After this pretreatment, the root tips were fixed in glacial acetic acid for 3 days, then they were placed in a solution of 1N-hydrochloric acid at 60° for 6 minutes for hydrolysis, transferred into Schiff's reagent and mounted in it for 2-4 hours. By this procedure, a small portion of the root tip becomes strongly stained, while the rest remains unstained. The stained portion was separated from the rest and squashed in a droplet of 1 per cent aceto-carmine.

The method described above gave good results for staining. Pretreatment for a longer time than 4 hours proved to be unfavourable.

---

* Contribution No. 322 from the National Institute of Genetics, Misima.

** Biological Institute, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya, Japan. Present address: National Institute of Genetics, Misima, Sizuoka Pref., Japan.

*** Some previous investigators treated buckwheat as belonging to *Polygonum*, but others treated it as *Fagopyrum*. In this paper it is referred to *Fagopyrum*.

The results are summarized in Table 1. and Figures 1–19.

The chromosomes of the species used in this study are mostly V– or J-shaped. Regarding this point, the author's results are in accord with the description of Löve and Löve[7].

Observation and Discussion

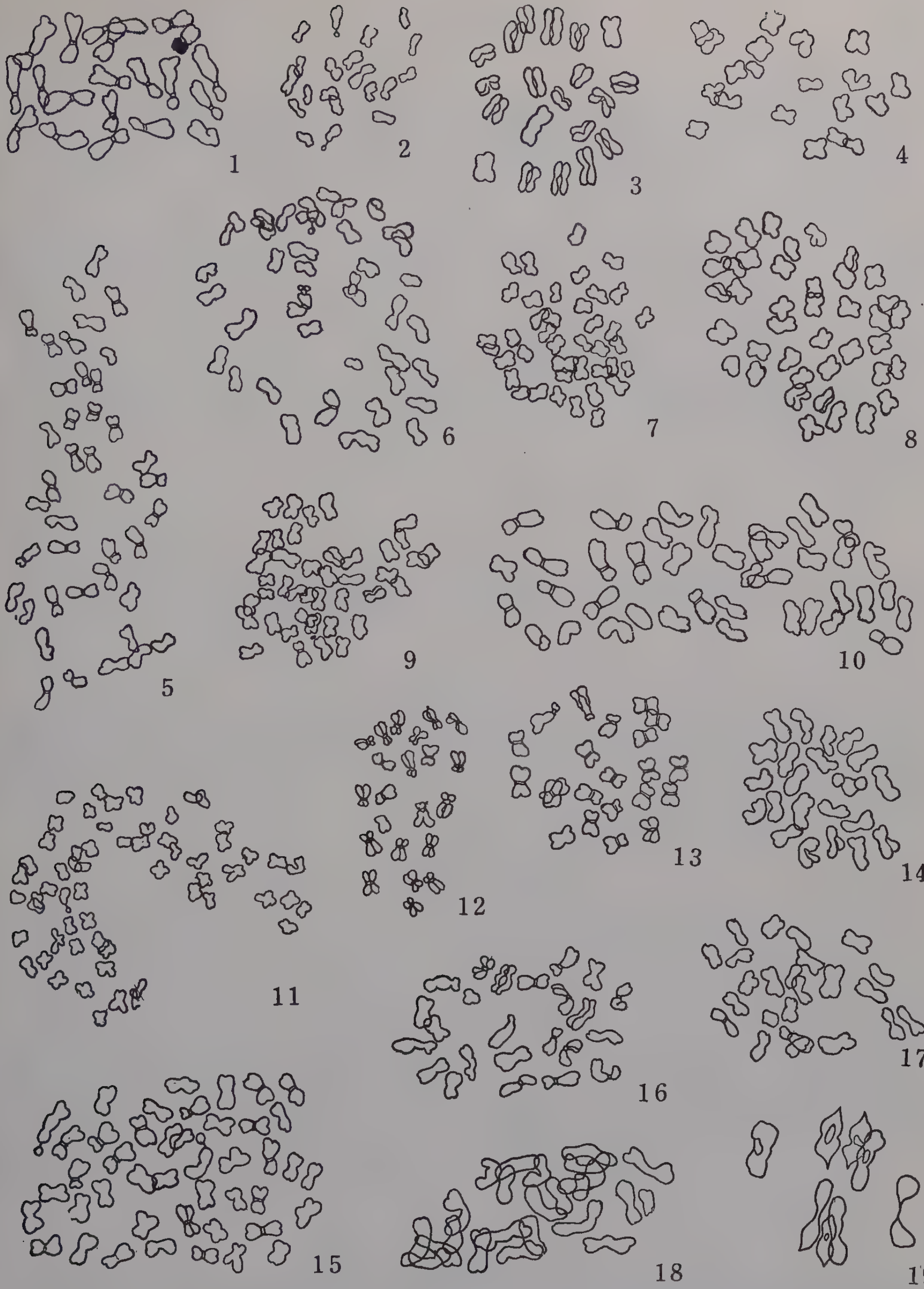
Table 1. The chromosome numbers of some *Polygonum* species

Species Name	Author's materials PMC (n)	Author's materials Root tip cell (2n)	Localities	Previous reporters	2n
Muehlenbeckia arisanensis Hay		20	Tokyo*		
Polygonum hydropiper Linn. var. *Maximowiczii* (Regel) Makino		20	Misima	Jaretzky (1928)	22, 24
				Sokolovskaja *et al.* (1938)	20
P. nipponense Makino		20	Misima		
P. weyrichii Fr. Schm. var. *alpinum* Maxim.		20	Mt. Fuji	Sugiura (1925)	20
P. yokusaianum Makino		40	Daiba		
P. blumei Meisn.		40	Misima		
P. persicaria Linn.		40	Misima	Jaretzky (1928)	44
P. tinctorium Aiton.		40	Tokyo*	Sugiura (1936)	40
P. japonicum Meisn.		40	Misima	Sugiura (1936)	44
P. thunbergii Sieb. et Zucc.		40	Misima	Sugiura (1936)	34
P. sieboldi Meisn.	20 (II)		Misima	Sugiura (1936)	34
P. aviculare Linn.		60	Misima	Löve & Löve (1948)	40, 60
P. nodosum Pers.		22	Misima	Löve & Löve (1942)	22, 44
P. orientale Linn.		22	Misima	Jaretzky (1928)	22
P. multiflorum Thunb.		22	Nagoya**	Suzuka (1950)	22
P. cuspidatum Sieb. et Zucc. (♀)		44	Misima	Jaretzky (1928)	c. 88
				Sugiura (1936)	44
P. perfoliatum Linn.		24	Misima		
P. bistorta Linn.		24	Mt. Ibuki	Jaretzky (1928)	44
				Sokolovskaja *et al.* (1938)	46
P. tenuicaule Bisset et Moore		24	Hakone		
Fagopyrum esculentum Moench	8 (II)	16	Nagoya	Jaretzky (1927)	16
F. tataricum Gaertn.	8 (II)	16	Nagoya	Sando (1939)	16

* These plants were supplied from Koishikawa Botanic Garden, Tokyo. The origin of the plants is unknown.

** These plants were supplied from Higashiyama Botanic Garden, Nagoya. The origin of the plants is unknown.

The chromosome numbers reported here are in several instances different from those reported by Jaretzky[1,2] and Sugiura[3]. *P. persicaria*, *P. japonicum*, *P. thunbergii* and *P. sieboldi* have 2n=40 according to the author's observation. But Jaretzky reported 2n=44 for *P. persicaria*, Sugiura found the same number in *P. japonicum* and reported 2n=34 for *P. thunbergii* and *P. sieboldi*. The author found 2n=24 in *P. bistorta*, while Jaretzky reported 2n=44 and Sokolovskaja *et al.*[13] counted 2n=46 in the same species. Some of these differences may be the result of aneuploidy.



Figs. 1–19. Chromosome of *Polygonum* species. Magnification: ca. 2150 ×

Fig. 1. *Muehlenbeckia arisanensis* Hay. Fig. 2. *Polygonum hydropiper* Linn. var. *Maximowiczii* (Regel) Makino Fig. 3. *P. nipponense* Makino Fig. 4. *P. weyrichii* Fr. Schm. var. *alpinum* Maxim. Fig. 5. *P. yokusaianum* Makino Fig. 6. *P. blumei* Meisn. Fig. 7. *P. persicaria* Linn. Fig. 8. *P. tinctorium* Aiton. Fig. 9. *P. japonicum* Meisn. Fig. 10. *P. thunbergii* Sieb. et Zucc. Fig. 11. *P. aviculare* Linn. Fig. 12. *P. nodosum* Pers. Fig. 13. *P. orientale* Linn. Fig. 14. *P. multiflorum* Thunb. Fig. 15. *P. cuspidatum* Sieb. et Zucc. Fig. 16. *P. perfoliatum* Linn. Fig. 17. *P. bistorta* Linn. Fig. 18. *P. tenuicaule* Bisset et Moore Fig. 19. *Fagopyrum tataricum* Gaertn.

All figures show somatic chromosome in root tip cells except Fig. 19 which shows meiotic chromosomes in pollen mother cells.

Regarding this point, Jaretzky[2]) reported aneuploid chromosome numbers in *P. filiforme*. But Sugiura[3]) did not find aneuploidy in this species. Whether aneuploidy is found in the species mentioned above, presents a problem for further studies. Meanwhile, it is assumed that the different chromosome numbers reported for some species are due to geographical variation.

As the basic chromosome number of the genus *Polygonum*, 10 and 11 have been assumed by previous investigators. Most of the species examined by the author have shown multiple numbers of 10 or 11 as described above. Furthermore, in three species: *P. perfoliatum*, *P. tenuicaule* and *P. bistorta*, 2n=24 were found. The first two species were examined cytologically for the first time. But for the last one, Jaretzky[2]) and Sokolovskaja *et al.*[13]) have reported 2n=44 and 2n=46 respectively. A basic chromosome number of 12, added to 10 or 11, could be assumed for such cases, although it is not safe to draw a definite conclusion without an examination of meiotic pairing.

Summary

Chromosome numbers of some species of *Polygonum* and related genera have been observed. The results are summarized in Table 1. In three species the basic number 12 has been found. This basic number was not yet reported up to date for Polygonaceae.

The author wishes to express his appreciation to Prof. Tamaki Shimamura of Nagoya University, and to Dr. Yô Takenaka, Head of Department of Cytogenetics, National Institute of Genetics, for their kind encouragements and advice during course of the present study. He also thanks to Dr. F. A. Lilienfelt, who was kind enough to make some corrections in the manuscript.

References

1) Jaretzky, R., Ber. dtsch. Bot. Ges. **45**: 48 (1927). 2) ——, Jb. wiss. Bot. **69**: 357 (1928). 3) Sugiura, T., Cytologia **7**: 544 (1936). 4) Löve, A., Hereditas **28**: 289 (1942). 5) ——, and Löve, D., Bot. Notiser **19** (1948). 6) ——, and ——, "Chromosome Numbers of Northern Plant Species". Reykuavik. (1948). 7) ——, and ——, Canad. Jour. Bot. **34**: 501 (1956). 8) Suzuka, O., Rept. Kihara Inst. Biol. Res. **4**: 57 (1950). 9) Darlington, C. D., and Janaki Ammal, E. K., "Chromosome Atlas of Cultivated Plants". George Allen & Unwin Ltd., London (1945). 10) Darlington, C. D., and Wylie, A. P., "Chromosome Atlas of Flowering Plants". George Allen & Unwin Ltd., London (1955). 11) Doida, Y., Bot. Mag. Tokyo **70**: 31 (1957). 12) ——, Ann. Rep. Nat. Inst. Genet. (Japan) **9**: 57 (1958). 13) ——, Bot. Mag. Tokyo **73**: 278 (1960).

摘　　要

土井田幸郎:　タデ属植物の細胞学的研究

タデ属植物における種の系統関係を論ずるために，タデ科のタデ属および近縁属植物の染色体数を調べた.

その結果，根端細胞で24の染色体数をもつ3種の植物を見出した．これら3種の基本染色体数は12と考えられる．タデ科植物においては，これまで基本染色体数は8, 10, 11および17が報告されているが[9,10]) 12という報告は本報が始めてである.（国立遺伝学研究所)

Bot. Mag. Tokyo, **37**; 341—347 (September 25, 1960)

Studies on the Light Controlling Flower Initiation of *Pharbitis Nil.* VII: Light-break

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

Received March 4, 1960

It is well known that a short interval of light (light-break) given during the dark period inhibits flower initiation of short day plants[1,2]. Generally, the light-break is most effective near the middle of the daily dark period. When the light-break is given during a long dark period of 36–72 hours' duration, it is effective only during the first 6 to 10 hours of darkness or towards the end of the dark period[3-7].

In a preliminary report, however, it was suggested that the light-break towards the end of the long dark period had little effect on the flowering response of *Pharbitis* seedlings[8].

Extensive studies have been made by Borthwick *et al.* on the action spectrum for the light-break, and it was found that red light is the most effective[9,10]. Furthermore, it was found that the inhibitory effect of red radiant energy was almost completely reversed by a subsequent irradiation with far-red[11,12].

In *Pharbitis*, too, red light is the most effective portion of the spectrum for the light-break, but recently Nakayama reported that the inhibitory effect of the red can not be reversed by a subsequent irradiation with far-red in *Pharbitis* seedlings. Furthermore far-red radiant energy is also effective for the light-break when given to the plant during the initial hours of dark period[13]. Thus, *Pharbitis* plants appear to differ from other short day plants in their response to the light-break.

In the present experiments, the light-break with red or far-red radiant energy was given to *Pharbitis* seedlings at various times during the long dark period, and Nakayama's experiments in which the antagonism between red and far-red was not observed were examined in detail.

Material and Methods

Material used was seedlings of *Pharbitis Nil*, strain "Violet". The experimental methods employed were similar to those described in a previous paper[14]. Red radiant energy was obtained from pink fluorescent lamps with a filter of 2 layers of red cellophane. Far-red was obtained from incandescent lamps with a filter composed of 5 cm. water, 2 layers of blue and 2 layers of red cellophane.

Experiments and Results

Experiment 1. One group of plants were irradiated for 2 minutes with red radiant energy of 1.5 kiloerg/cm.^{2}/sec. at various times during a 48-hour dark period. Another group was treated in the same way as the first except that 5 minutes of far-red followed the 2-minute irradiation with red.

Results are summarized in Fig. 1. Light-break with red was effective only 8 to 12 hours after the beginning of the dark period. The red irradiation had no effect

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

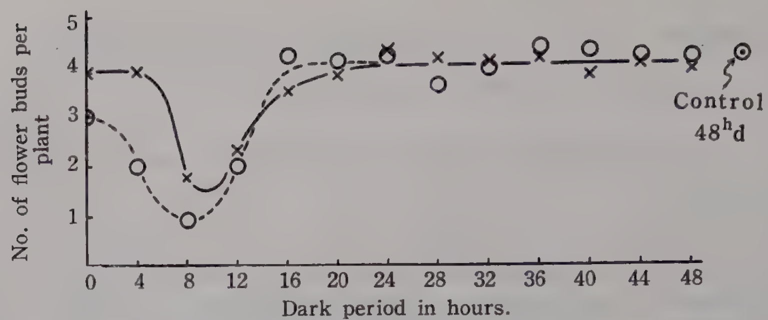


Fig. 1. Flowering response of *Pharbitis* seedlings subjected to 2-minute red (1500 erg/cm.^{2}/sec.) and that followed by 5-minute far-red (15 kiloerg/cm.^{2}/sec.) at various times during a 48-hour dark period.
Solid line: red
Broken line: red followed by far-red
(Treated on March 17 and dissected on April 1, 1959)

when given towards the end of the dark period. A far-red irradiation immediately following the red did not remove the inhibitory effect of the red at any time during the dark period, but it intensified the flower-inhibitory effect during the first 12 hours. This result agrees with the findings of Nakayama[13]).

Experiment 2. One group of the plants were irradiated for 5 minutes with far-red radiant energy of 15 kiloerg/cm.^{2}/sec. at various times during the 48-hour dark period. Another group was treated in the same way, but 2 minutes of red radiant energy followed the 5-minute exposure to far-red.

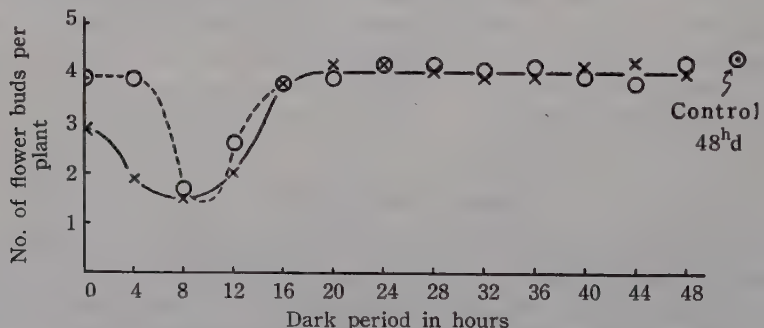


Fig. 2. Flowering response of *Pharbitis* seedlings subjected to 5-minute far-red (15 kiloerg/cm.^{2}/sec.) and that followed by 2-minute red (1500 erg/cm.^{2}/sec.) at various times during a 48-hour dark period.
Solid line: far-red
Broken line: far-red followed by red
(Treated on March 18 and dissected on April 3, 1959)

As shown in Fig. 2, far-red given during the first hours of the dark period inhibits flower initiation. Maximum inhibition was observed after 8 hours of the darkness. Red radiant energy following the far-red irradiation completely reversed the flower-inhibitory effect of far-red at the beginning or the 4th hour of the dark period, but had little effect at any other times.

Experiments 1 and 2 were repeated several times with dark periods of 16, 24 or 32 hours. In all experiments, the light-break with red radiant energy was effective only after 8 to 12 hours of darkness and far-red acted like red only during the first 12 hours. Far-red following a red irradiation, and red following a far-red irradiation showed similar effects in all experiments.

Experiment 3. One minute of red radiant energy of 1500 erg/cm.^{2}/sec. was given in the middle of a 16-hour dark period, and far-red irradiations (15 kiloerg/cm.^{2}/sec.) of various durations were given following the red irradiation. Results are shown in Table 1.

Table 1. Flowering response of *Pharbitis* seedlings irradiated for 1 minute with red radiant energy of 1500 erg/cm.^{2}/sec. followed by various durations of far-red irradiation (15 kiloerg/cm^2./sec.) in the middle of a 16-hour dark period. (Treated on March 28 and dissected on April 16, 1959)

Duration of far-red irradiation	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant
Dark control	38	100	3.1
0	37	51.3	0.6
1″	37	62.2	0.8
5″	38	50.0	0.8
10″	36	58.3	0.8
30″	37	16.2	0.2
1′	37	29.8	0.3
2′	37	2.7	0.0
5′	37	0	0

A far-red irradiation for 1–10 seconds had no significant effect on flowering response, but irradiations of 30 seconds to 5 minutes intensified the flower-inhibitory effect.

Experiment 4. One minute of red and 1 minute of far-red were given alternately in the middle of the 16-hour dark period. As shown in Table 2, it appears that the inhibitory effect of the red irradiation is not reversed by a subsequent irradiation

Table 2. Flowering response of *Pharbitis* seedlings exposed alternately to 1-minute red and 1-minute far-red irradiation in the middle of a 16-hour dark period.
R: red radiant energy of 1500 erg/cm.^{2}/sec.
FR: far-red radiant energy of 15 kiloerg/cm.^{2}/sec.
(Treated on March 10 and dissected on March 27, 1959)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant
Dark control	37	100	4.1
R	37	91.9	2.0
FR	37	86.5	2.1
R→FR	36	52.8	0.8
R→FR→R	31	67.8	1.2
R→FR→R→FR	35	0	0
R→FR→R→FR→R	37	27.0	0.3
R→FR→R→FR→R→FR	37	0	0

with far-red, although the inhibitory effect of far-red is partially reversed by a subsequent exposure to red. This result does not agree with the results obtained with *Xanthium*, Biloxi soybean, etc., in which the inhibitory effect of red radiant energy is reversed by a subsequent irradiation with far-red.

Experiment 5. One minute of red and 10 seconds of far-red, or 2 minutes of far-red and 20 seconds of red were given alternately in the middle of a 16-hour dark period. Results are presented in Table 3.

Table 3. Flowering response of *Pharbitis* seedlings exposed alternately to 1-minute red and 10-second far-red irradiation or 2-minute far-red and 20-second red irradiation in the middle of a 16-hour dark period.
(Treated on April 6, and dissected on April 25, 1959)

Group	Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant
1	dark control	38	100	2.9
	R	37	81.1	1.1
	FR	35	100	3.0
	R→FR	34	91.2	1.5
	R→FR→R	36	55.6	0.6
	R→FR→R→FR	36	63.9	0.8
	R→FR→R→FR→R	34	32.4	0.3
	R→FR→R→FR→R→FR	34	76.5	0.8
2	FR	35	85.5	1.1
	R	38	84.2	1.3
	FR→R	35	74.3	0.8
	FR→R→FR	35	51.5	0.5
	FR→R→FR→R	35	62.9	0.7
	FR→R→FR→R→FR	35	2.9	0.0
	FR→R→FR→R→FR→R	33	30.3	0.3

Group 1 R: 1-minute red (1500 erg/cm.^{2}/sec.)
FR: 10-second far-red (15 kiloerg/cm.^{2}/sec.)
Group 2 F: 20-second red (1500 erg/cm.^{2}/sec.)
FR: 2-minute far-red (15 kiloerg/cm.^{2}/sec.)

Ten seconds of far-red given in the middle of 16-hour dark period had little effect on the flowering response, although such far-red radiant energy can partially reverse the inhibitory effect of red.

If 2 minutes of far-red was followed by a 20 second exposure to red, the flowering response was reduced, but when both red and far-red are given alternately some reversing effect of red is observable. That is, the rate of repromotion appears to increase with repetition of cycles.

Experiment 6. Plants were placed in darkness for 8 hours. At the close of the dark period red or far-red radiant energies of different intensities were given for various durations so as to make the total energy 180 kiloerg/cm.^{2} or 1800 kiloerg/cm.^{2} respectively. Immediately thereafter, plants were again placed in darkness for 16 hours.

Results are shown in Table 4. Both red and far-red inhibited flower initiation more vigorously when given at low intensities for long durations.

Table 4. Influence of light intensities upon effectiveness of light-break.
Red or far-red radiant energies of various intensities were inserted between 8- and 16-hour dark period for various durations, so as to make the total energy 180 or 1800 kiloerg/cm.², respectively.
(Treated on April 25 and dissected on May 9, 1959)

Light inserted			No. of plants dissected	% of plants with flower buds	No. of flower buds per plant
Quality	Duration	Intensity in erg/cm.²/sec.			
R	2′	1500	35	80.0	1.4
R	5′	600	37	78.4	1.4
R	30′	100	35	68.6	1.0
R	2h	25	38	0	0
R	4h	12.5	37	0	0
R	8h	6	34	2.9	0.0
FR	2′	15000	34	100	2.7
FR	5′	6000	32	100	2.7
FR	30′	1000	37	45.9	0.5
FR	2h	250	37	37.8	0.4
FR	4h	125	37	16.2	0.2
FR	8h	63	38	13.2	0.1
dark control			38	100	4.0
32h R (6 erg/cm.²/sec.)			34	2.9	0.0
32h FR (63 erg/cm.²/sec.)			29	0	0

Discussion

In *Pharbitis* seedlings a light-break with red radiant energy is effective only 8 to 12 hours after the beginning of the dark period. The process inducing flowering probably becomes light-sensitive at this time, and thereafter, the process becomes stable to the light.

A brief far-red irradiation given during the first 12 hours of the dark period also inhibits flower initiation of *Pharbitis Nil*. As had been reported previously[15]), far-red preceding the inductive dark period also inhibits flowering especially when the far-red irradiation lasts for 8 hours or more. The present experiments showed that the flower-inhibitory effect of far-red is at a maximum near the 8th hour of the dark period. The first process of the inductive dark period is believed to proceed under far-red irradiation[15,16,17]). Therefore, it is conceivable that, if the far-red irradiation is continued for 8 hours or more preceding the dark period, the first process of the inductive dark period proceeds under this light, and the process becomes sensitive to far-red, and subsequently heavy flower inhibition takes place.

Low-intensity light is also effective for light-break (cf. Experiment 6). Hitherto, an inhibitory effect of low-intensity light preceding the inductive dark period was considered due to the deficiency of photosynthate and was completely overcome by feeding sugar. Preliminary experiments, however, showed that the flower-inhibitory effect of low-intensity light preceding the dark period is not overcome by feeding sugar in *Pharbitis* plants (unpublished). Furthermore, as has been reported previously: 1) the first process of the inductive dark period can proceed under low-intensity

light (10 lux)[16,17]), 2) low-intensity light of less than 8 hours preceding the inductive dark period does not inhibit flower initiation if it contains little far-red, but that of 8 hours or more inhibits flowering[14-17]), 3) a brief red irradiation following the 8-hour low-intensity light period (preceding the dark period) inhibits flower initiation[17]). Therefore, it seems probable that low-intensity light of long duration preceding the dark period has a light-break effect. That is, during the first 6–8 hours of the low-intensity light, the first process of the inductive dark period proceeds[16,17]) and the following low-intensity light period gives the light-break effect.

In *Pharbitis* seedlings flower inhibition caused by a red interruption in the middle of 16-hour dark period appears not to be reversed by the following far-red irradiation of 1 minute or more. The far-red radiant energy may reverse the flower-inhibitory effect of red to some extent, but the flower-inhibitory effect of far-red—the mechanism appears to differ from that of the red interruption—may exceed the reversing one. As shown in Experiment 5, if the duration of the far-red irradiation was shortened to 10 seconds, some reversing effect is apparent. The reversing effect of the far-red may exceed the inhibitory one in this case. On the other hand, if the duration of the red irradiation was shortened to 20 seconds, some reversing effect of red on the far-red response was observed.

Red radiant energy given at the 4th hour of the dark period does not inhibit flower initiation, but far-red given at this time does so. The inhibitory effect of the latter can be reversed completely by a following red irradiation (cf. Experiment 2). The red-far-red antagonism is observed clearly in this case.

The flower-inhibitory effect of red and far-red radiant energy is assumed to be based on different mechanisms, although the antagonism between them exists in *Pharbitis* seedlings. Nakayama reported that in *Pharbitis* seedlings no repromotive action of a far-red irradiation immediately following the red one was observed. In his experiments, the intensity of far-red was so high that the inhibitory effect of far-red may have exceeded the reversing one.

In other short day plants, such as *Xanthium*, Biloxi soybean etc., far-red may have little effect on flowering when given in the middle of the dark period, and the reversing effect of far-red may be observed more clearly.

Summary

1) A red interruption was effective for flower inhibition only 8 to 12 hours after the beginning of the dark period irrespective of the duration of the dark period. A 5-minute exposure to far-red (15 kiloerg/cm.^{2}/sec.) given immediately following a 2-minute red irradiation (1500 erg/cm.^{2}/sec.) did not remove the inhibitory effect of red at any time during the dark period.

2) A brief far-red irradiation given during the first 12 hours of the dark period inhibited flower initiation. Maximum inhibition was obtained at the 8th hour. Two minutes of red following 5 minutes of far-red reversed the inhibitory effect of far-red completely during the first 4 hours of the dark period, but had little effect at any other time.

3) The inhibitory effect of 1-minute red irradiation given in the middle of a 16-hour dark period was reversed to some extent by the following far-red irradiation, if the duration of the latter was shortened to some 10 seconds.

The inhibitory effect of 2-minute far-red irradiation given in the middle of a 16-hour dark period was reversed to some extent by a following 20-second exposure to

red.

4) The flower-inhibitory effect of red and far-red given at the 8th hour of a long dark period is stronger when given to the plant with low intensities for long durations than when given with high intensities for short durations.

Grateful acknowledgment is given to Prof. S. Imamura for his suggestion and criticisms.

References

1) Hamner, K. C., and Bonner, J., Bot. Gaz., **100**: 388 (1938). 2) Harder, R., und Bode, O., Planta, **33**: 469 (1943). 3) Salisbury, F. B., and Bonner, J., Plant Physiol., **31**: 141 (1956). 4) Wareing, P. F., Physiol. Plantarum, **7**: 157 (1954). 5) Bünsow, R., Z. Bot., **41**: 257 (1953). 6) Melchers, G., Z. Naturforsch., **11** b: 544 (1956). 7) Schwabe, W. W., Physiol. Plantarum, **8**: 263 (1955). 8) Takimoto, A., and Ikeda, K., Bot. Mag. Tokyo, **73**: 91 (1960). 9) Parker, M. W., Hendricks, S. B., Borthwick, H. A., and Scully, N. J., Bot. Gaz., **108**: 1 (1946). 10) Borthwick, H. A., Hendricks, S. B., and Parker. M. W., Bot. Gaz., **110**: 103 (1948). 11) ——. ——, and ——, Proc. Natl. Acad. Sci., **38**: 929 (1952). 12) Downs, R. J., Plant Physiol., **31**: 279 (1956). 13) Nakayama, S., The Science Report of the Tohoku Univ., Sendai, S 4, **24**: 137 (1958). 14) Takimoto, A., and Ikeda, K., Bot. Mag. Tokyo, **72**: 137 (1959). 15) ——, and ——, ibid. **72**: 181 (1959). 16) ——, and ——, ibid. **72**: 388 (1959). 17) ——, and ——, ibid. **73**: 175 (1960).

摘　要

滝本　敦・池田勝彦:　アサガオの花芽形成を支配する光条件について VII:　光中断

1) 暗期の長さに関係なく，赤色光による光中断は暗期開始後 8～12 時間目においてのみ有効である．光中断として 2 分間赤色光 (1500 erg/cm.²/sec.) を与えた直後に 5 分間近赤外光 (15 kiloerg/cm.²/sec.) を照射しても，前者による花芽形成抑制効果は消却されない．

2) 暗期の長さに関係なく近赤外光による光中断は暗期開始後 0～12 時間目においてのみ有効であり，8 時間目で最もいちじるしい花芽形成抑制効果を示す．5 分間の近赤外光照射に続いて 2 分間赤色光を与えると，暗期の初期 0～4 時間目においては，前者による花芽形成抑制効果が完全に消却されるが，暗期の他の時期においては，ほとんどその影響が見られない．

3) 16 時間暗期の中央で 1 分間の赤色光と 10 秒間の近赤外光を交互に数回与えると，最後の照射光が赤色光の場合よりも，近赤外光の場合の方が高い開花反応を示す．これに反して，2 分間の近赤外光と 20 秒間の赤色光を交互に与えた場合には，最後に与えた光が近赤外光の場合よりも赤色光の場合の方が高い開花反応を示す．

赤色光と近赤外光の拮抗作用はあるが，各々が異なった機構で花芽形成を抑制するため，拮抗作用がないように見える場合が多いものと考えられる．

4) 赤色光，近赤外光共に強い光を短時間照射するよりも，弱い光を長時間照射する方が強い花芽形成抑制作用を示す．(京都大学農学部応用植物学研究室)

Bot. Mag. Tokyo, **73**: 348–354 (September 25, 1960)

Studies on Adventitious Bud Formation
(I) Morphological and Histological Observations on the Adventitious Buds on Tomato Leaves

by Kayô FUKUMOTO*

Received March 5, 1960

Adventitious bud formation on the leaf edge in *Bryophyllum* and *Woodwardia orientalis* Sw. is a well known fact. In other kinds of plants such a phenomenon, without special operations as cutting etc., can seldom be seen. Several years ago, when the author was engaged in grafting experiments on tomato plants, he found many adventitious buds and shoots formed on leaf rachises of stock-plants (variety Red Cherry) which were fully manured. Recently a similar phenomenon was reported by V. P. Rozhdestvensky (1958)[1]. He made detailed external observations on the bud formation, but no anatomical or histological research on it was made by him. In order to investigate this phenomenon more profoundly, the present author carried out in 1959 some field experiments and laboratory observations. The results are described in this paper.

Material and Methods

Three tomato varieties—Red Cherry, Yellow Pear and Jubilee—were used as the material. Seedlings raised in the nursery-bed were planted on the experimental field in June. Abundant manure and chemical fertilizers were put in the field at the time of transplantation. Additional fertilization was made several times during the growing period. A few plants of the variety Red Cherry were planted in flower-pots with small doses of fertilizers given and were left to themselves without any trimming or pinching. These plants were used as a control. Experimental plants on the field were brought up by the common method of cultivation with propping and trimming. All lateral shoots on the stem were nipped off as they came out. The experimental plants belonging to the variety Red Cherry were divided into two parts. In the first part only the lateral shoots were removed, while in the second part the plants were pinched at a height of about 1.5 m. when adventitious buds appeared on their leaves and all flower buds and young fruits borne on them were also removed.

For anatomical and histological studies, portions of the leaf rachises, where adventitious buds appeared or about to appear, were cut off and fixed with 96% ethanol or Carnoy's fluid. Preparations for microscopy were made by the paraffin method. Sections were cut 15 or 20 μ thick and stained by Heidenhain's hematoxylin or gentian violet.

Results

The experimental plants showed some symptoms of superfluous nutrition during the vegetation period. Leaf blades became thicker and somewhat fleshy and bent

* Biological Laboratory, Faculty of General Education, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan.

upwards at the margin. On the cut-surfaces of lateral shoots and floral axes were formed large calluses, and abundant callus-buds appeared on them. To avoid waste of nutrient substances, these callus-buds were also cut off as they appeared. Such phenomena as mentioned above were not seen on the control plants grown in flower-pots. Early in July, when the rainy season was over, adventitious buds began to appear on the leaf rachises of the experimental plants of Red Cherry and Yellow Pear. In Jubilee adventitious bud formation was observed about 20 days later. No adventitious bud was formed on the control plants. On August 5 the number of the adventitious buds were taken for each experimental plant. Tables 1 and 2 show the state of adventitious bud formation in Red Cherry and Jubilee, respectively. As shown in the tables, adventitious buds are formed only on the middle and upper leaves on the stem. Old bottom leaves and young top leaves never form adventitious buds. On shade leaves they are seldom seen. The portions where adventitious buds are initiated are strictly confined to the axillary parts of petiolules on the leaf rachis (Fig. 1). In other parts of the rachis adventitious buds are never formed.

Bud formation begins at first in the proxymal part of the leaf rachis and gradually proceeds toward the distal part. 1–3 buds usually appear on one leaf in Red Cherry and Yellow Pear, while in Jubilee as

Table 1. The state of adventitious bud formation in Red Cherry (August 5)

Leaf order*	Experimental plants									Total
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	
17	1									1
18	3							1	1	5
19	0			2		2		0	0	4
20	2		3	2	1	0		2	2	12
21		1	0	3	2	3	2	3	3	17
22		1	1	1	1	0	3	1	2	10
23		2	1		1	0	2		0	6
24			1			1	2		2	6
25						1	0			1
26							1			1
Total	6	4	6	8	5	7	10	7	10	63

* Number was given from lower to upper.

Table 2. The state of adventitious bud formation in Jubilee (August 5)

Leaf order*	Experimental plants				Total
	No. 1	No. 2	No. 3	No. 4	
13		1			1
14		4	4		8
15	1	4	3	4	12
16	1	4	3	6	14
17		2	4	7	13
18		5	2	2	9
19		4	1	4	9
20		1	3	3	7
21		2	3	4	9
22		4	0	2	6
23			3	2	5
24				1	1
Total	2	31	26	35	94

* Number was given from lower to upper.

many as 5–7 buds can be observed. Of all the buds formed only one or two continue to grow and the rest remain undeveloped.

Figs. 1–4. Adventitious buds and shoots on tomato leaves. 1, bud primordia on a leaf (shown by arrows). 2, a flowering adventitious shoot. 3, two fruit-bearing adventitious shoots on a leaf. 4, sketch of the basal part of the adventitious shoots shown in Fig. 3. S, stem; L, compound leaflet; l, simple leaflet.

External morphology of the adventitious buds: The first sign of adventitious bud formation is shown by a slight rising on the leaf rachis. This sphere of rising is 3–5 mm. in diameter and is easily distinguished from the other part of the rachis by its pale yellowish colour. Before long a small projection or protuberance appears in the centre of this sphere (Fig. 5–A) and a few minute primitive leaves are formed about it (Fig. 5–B, C). These leaves have no leaf blade and present an appearance of a small mushroom. At this stage no distinct stem can be seen from the outside. At the next stage the basal part of the bud grows upwards into a short stem bearing several incomplete simple leaves on it (Fig. 5–D). The process of adventitious bud formation up to this time is rather laggard and many of the buds stop their growth at this stage.

Rozhdestvensky reported that he had observed a tuberous basal part of the adventitious bud. The present author, however, did not find such a phenomenon. After this stage some of the buds grow up into shoots with full-developed leaves, of which the lower ones are of simple structure and the upper of compound structure. Under better conditions such adventitious shoots form the first *inflorescence* on their stem near the 5th or 6th true leaf. On the plants of Red Cherry belonging to the second experimental part, of which the tops, flower buds and young fruits had been

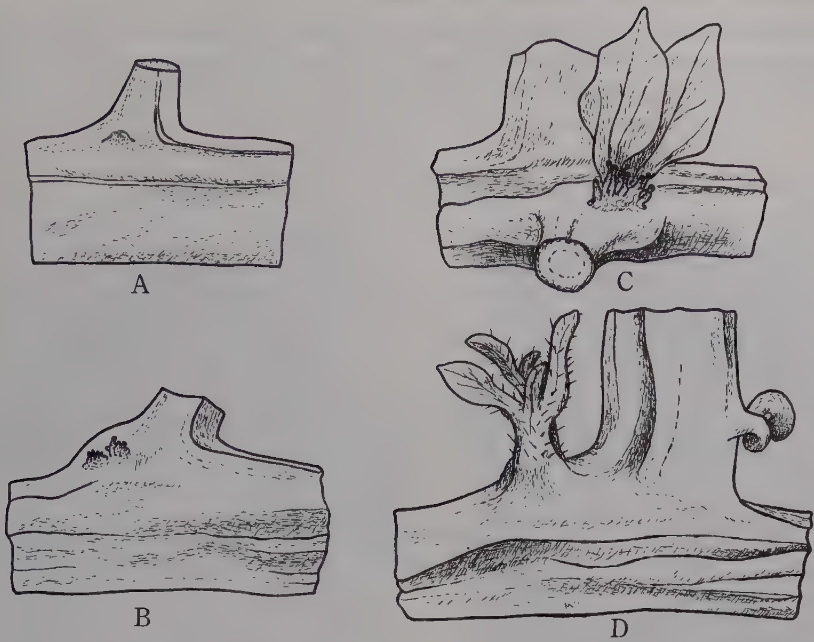


Fig. 5. Drawings to show stages in the development of an adventitious bud on the leaf rachis.

removed, some of the adventitious shoots bore a few fruits before the end of August (Fig. 3). On the other experimental plants which had been left with their primary fruits on the stem no flower of the adventitious shoots developed into fruit.

Anatomical and histological observations: Prior to the adventitious bud formation, subepidermal parenchymatous cells and even epidemal cells in the axillary parts of the petiolules begin to divide (Figs. 6, 7), forming a new tissue composed of one

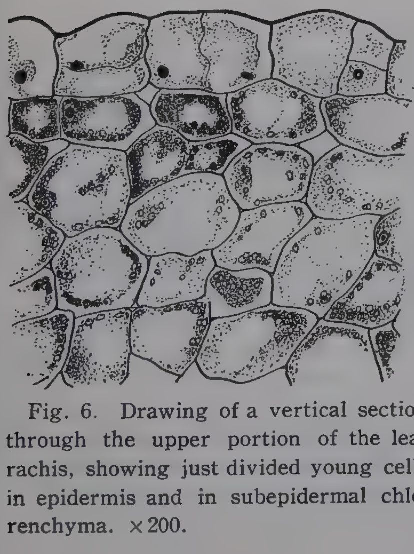


Fig. 6. Drawing of a vertical section through the upper portion of the leaf rachis, showing just divided young cells in epidermis and in subepidermal chlorenchyma. $\times 200$.

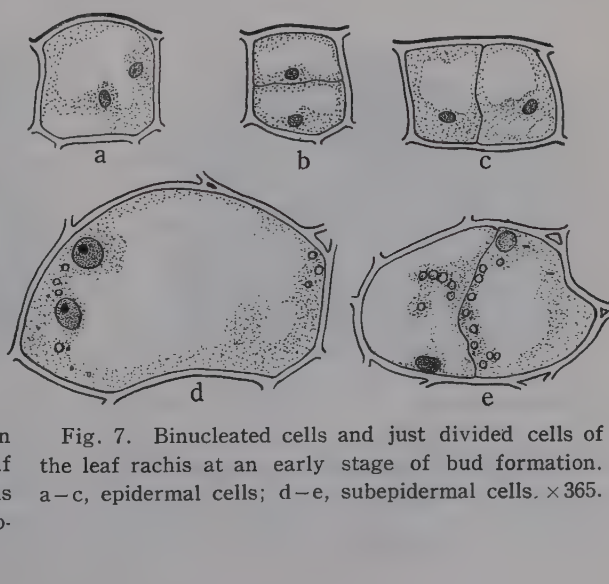


Fig. 7. Binucleated cells and just divided cells of the leaf rachis at an early stage of bud formation. a–c, epidermal cells; d–e, subepidermal cells. $\times 365$.

or two layers of small cells beneath the epidermis. These cells are rich in cytoplasm and colourless plastids (cf. Crooks[2]) and can be deeply stained by dyes (Fig. 8-A). In younger leaves such "rejuvinated" tissue is found only just under the epidermis of the axillary risings, while in older leaves it can be seen all around the rachis.

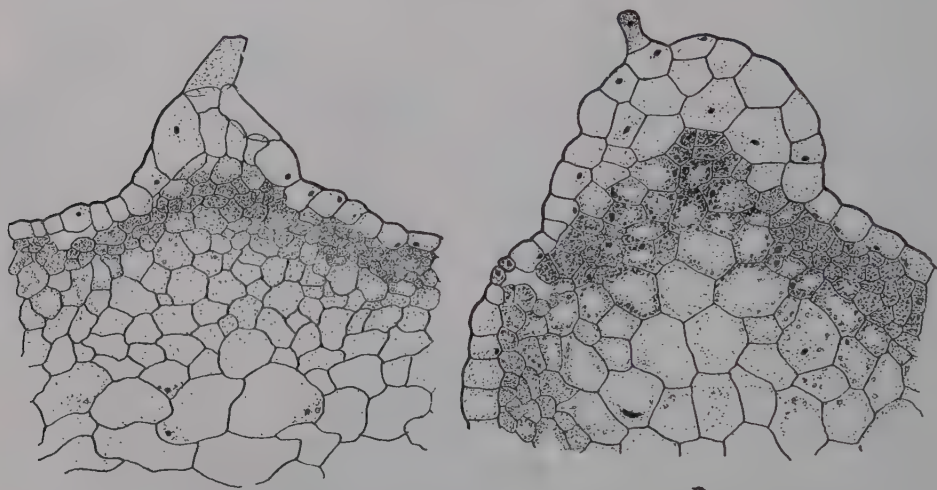


Fig. 8. Drawings of vertical sections through the upper portion of the leaf rachis, showing the protuberance in which a growing point is beginning to be formed. A, early stage; B, later stage. ×125.

In the centre of the rising these small cells continue to divide and by this process a dome-shaped "nidus" of embryonal cells is formed here (Fig. 8–B). As cell divisions go on, this portion of the subepidermal tissue swells out into a bud primordium on the surface of the leaf rachis (Fig. 9). After a while a few leaf primordia are developed about the protuberance (Fig. 10). The first procambia

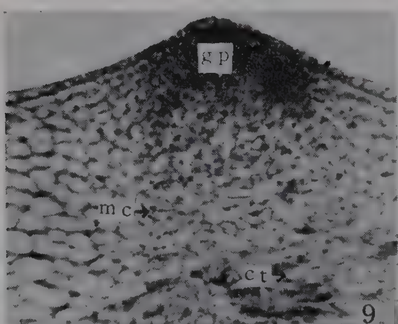


Fig. 9. Photomicrograph of a longitudinal section of a bud primordium. gp, growing point; mc, meristematic cells formed in parenchyma; ct, conducting tissue of the mother leaf. × 108.

Fig. 10. Photomicrograph of a median longitudinal section of an adventitious bud. gp, growing point; lp, leaf primordium; pc, meristematic tissue or procambium of the bud; ct, conducting tissue of the mother leaf. ×40.

appear in the leaf primordia before any vascular connexion between the bud axis and the stele of the rachis is established. At this stage a typical vegetative cone is formed at the apex of the bud primordium.

Meanwhile, at the bottom of the newly formed meristematic zone, deep seated parenchymatous cells of the rachis divide successively, forming the procambial strands which gradually extend towards the conducting tissue of the rachis (Figs. 9, 10). In Figs. 11 and 12 is shown the meristematic tissue or procambium just

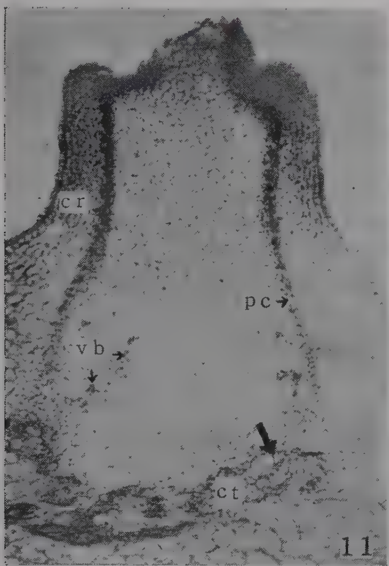


Fig. 11. Photomicrograph of a longitudinal section of the basal part of an almost completed adventitious bud, showing the meristematic tissue connected with the vascular system of the leaf rachis. cr, cortex of the bud; pc, procambial strand; vb, newly formed vascular bundles; ct, conducting tissue of the mother leaf. ×84.

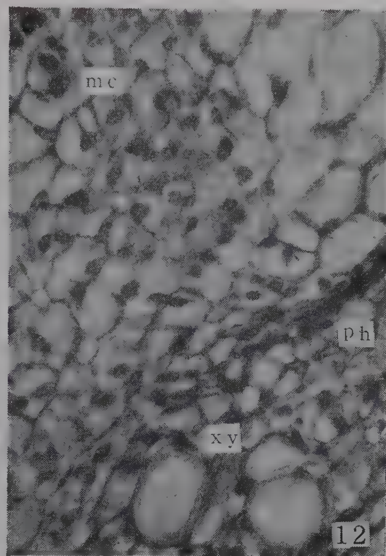


Fig. 12. Highly magnified photograph of the portion shown in Fig. 11 by an arrow. mc, meristematic cells; ph, phloem; xy, xylem. ×512.

attached to a primary vascular bundle of the mother leaf. In Fig. 11 newly formed vascular bundles are also seen in the lower part of the procambial strands. At later stages of development, vascular system of the adventitious shoot is observed completely jointed with that of the leaf rachis, although the arrangement of the vascular bundles near the jointing point is very irregular. Similar mode of conducting tissue formation in adventitious buds was observed in the hypocotyl of flax by D. M. Crooks (1933)[2], in the root-tuber of sweet-potato by K. Yasui (1944)[3] and in the stem-callus of lime etc. by V. N. Jultsev (1955)[4].

Consideration

Adventitious bud formation in regenerating tissues and organs has been reported by many authors. But the instances on tomato leaves as found by Rozhdestvensky and the present author are rather rare, and it is expected that this finding may serve as a clue to throw light on the mechanism of the normal organogenesis in higher

plants. The present report only deals with the results of morphological observations on the adventitious bud formation. The most interesting questions such as the cause of the adventitious bud formation on the leaf rachis or the reason of the restriction of the position of the formation within the axile of the leaflet are not touched on in this report. To answer these questions, more critical and experimental studies must be carried out. For the present only a supposition can be put forward that some hormone-like substance in collaboration with other material substances stimulates the bud initiation, and that the positional relation of the tissue or the organ concerned also plays an important role in it. Concerning the latter problem, it will be worthwhile to refer to Yasui's opinion that "any embryonal cell of a plant can be developed into an organ of a plant if it were placed in a suitable situation in which the very organ should develop under the normal condition".

Summary

1. Vigorous adventitious bud formation was observed on the leaf rachises of the tomato plants abundantly fertilized and systematically trimmed.

2. Adventitious buds appear only in the axillary parts of the petiolules of the middle and upper leaves.

3. Under better conditions some of the buds develop into fruit-bearing shoots.

4. Anatomical and histological investigations of the bud formation were made.

5. Growing point of an adventitious bud is initiated in the subepidermal tissue of the leaf rachis as a small group of meristematic cells, which are produced by cell divisions of the subepidermal chlorenchyma.

6. An adventitious bud appears at first as a small protuberance on the leaf rachis.

7. A few leaf primordia and a typical vegetative cone are formed on the protuberance.

8. Somewhat later the basal part of the bud grows longitudinally, and thus an adventitious shoot is formed.

9. By cell divisions in the depth of the parenchyma the procambial strands extend downwards to connect with the conducting tissue of the leaf rachis.

10. At later stages of development, the newly formed vascular system of the bud is seen completely jointed with that of the leaf rachis.

References

1) Rozhdestvensky, V. P., Bjulleten' Moskov. Obshch. Ispyt. Prir. Otd. Biol. **63** (5): 83 (1958). 2) Crooks, D. M., Bot. Gaz. **95**: 209 (1933). 3) Yasui, K., Proc. Imp. Acad. Tokyo **20**: 41 (1944). 4) Jurtsev, V. N., Trudy Prik. Bot. Genet. i Selek. **32**: 134 (1955).

摘　　要

福本日陽: 不定芽形成にかんする研究 (I) トマトの葉上不定芽の形態・組織学的観察

強度の肥培と摘芽などによって栄養過剰の状態におかれたトマトの葉の葉軸上に多くの不定芽が生じ，それらのうちのあるものは開花結実するにいたった．不定芽は中部の葉の小葉の腋部にかぎって生じ，一枚の葉に数個ずつみられた．

不定芽のできはじめは，小葉の腋部の表皮下柔細胞の分裂による胚的細胞の小群としてみられる．のちにその部分が上方に隆起して，まわりに葉原基，中央に生長内錐を形成する．

芽の下方の柔細胞も分裂をはじめ，それによってつくられた原維管束が葉軸の管束の方に向かってのびてゆく．通導組織ははじめ葉原基内にできるが，次第に下部の方にもつくられ，ついに葉軸のそれと結合するにいたる．不定芽のできる原因についてはくわしいことはしらべられていない．（東京農工大学一般教育部生物学研究室)

Bot. Mag. Tokyo, **73**: 355—359 (September 25, 1960)

Biochemical Studies on Calcareous Algae
1. Major Inorganic Constituents of Some Calcareous Red Algae*

by Kurazo FURUYA**

Received March 7, 1960

The red algae belonging to Corallinaceae have been known to contain a remarkably large amount of calcium chiefly in form of its carbonate[1-5]). Also in some members of Chaetangiaceae (*Galaxaura*), Squamariaceae (*Peyssonnelia*) and others the deposition of calcium takes place, though to a lesser extent[6]). While there are no essential differences between calcareous and non-calcareous red algae in respect of the mode of reproduction as well as of the chemical nature of storage substances as floridean starch[7-9]) or cell wall constituents such as cellulose[7,9,10]) and galactan sulfate[7,11-13]), the lime incrustation of calcareous algae represents a striking contrast to other red algae. The calcareous algae grow side by side with non-calcareous algae under the same habitat conditions. In view of these situations it may be inferred that in the calcareous algae some particular metabolism is operative in connection with the specific calcium deposition.

Together with lime magnesium carbonate has also been known to occur in Corallinaceae[1,5]), but its content has hitherto been estimated for relatively limited species. It will be interesting to see whether or not the content of magnesium in calcareous algae is related in some way with that of calcium.

As to the inorganic anions other than carbonate, sulfate and phosphate may be considered important. Sulfate is an essential constituent of the intercellular substance of the red algae, presumably corresponding to the pectin of higher plants, and confers acid property on this substance. Phosphate was suggested by Roche *et al.*[13]) to take some part in the calcification of calcareous algae analogous to the ossification in animals.

The present series of investigations has been undertaken as an ultimate aim to give some information about the mechanism of lime deposition in calcareous algae. In this paper the author will report as a preliminary step, the results of the determination of some inorganic constituents of various calcareous algae together with some microscopic observations of the initial stage of calcification.

Materials and Methods

In the present study analyses were made with eight species belonging to Corallinaceae and three species belonging to Chaetangiaceae. Most of them were collected during March to July, near the Shimoda Marine Biological Station of the Tokyo University of Education. The collected materials were washed thoroughly with distilled water until they were free of soluble chloride and air-dried. Prior to use they were powdered.

Total amount of ash was estimated by incineration of each material at 500 to 550° for about 10 hours. With the ash thus obtained the determination of calcium, magnesium, sulfate and phosphate was carried out. Separation of calcium and

---

* Contribution from the Shimoda Marine Biological Station, No. 102.

** Biological Institute, Tokyo Gakugei University, Tokyo, Japan.

magnesium was made in the usual way. Calcium was estimated by precipitation as Ca–oxalate followed by titration with permanganate. Magnesium was isolated as magnesium ammonium phosphate and weighed in the form of magnesium complex of oxine[15]). In calcareous algae, as in other red algae, a part of sulfate is present as polysaccharide sulfate and the sulfuric acid of this type is partly lost by incineration. Accordingly the determination of total sulfate, the sum of inorganic and ester sulfate, was made by boiling the sample with 12 per cent hydrochloric acid and weighing the sulfate in the digest as barium sulfate. Carbonate was estimated by the liberation of carbon dioxide by addition of 5 per cent hydrochloric acid at room temperature and absorption in soda lime.

The sections for microscopic examination were prepared by the procedure used in mineralogy, as the thallus of calcareous algae is heavily incrusted with lime rendering the use of conventional microtome impossible. With *Joculator maximus* two sorts of the sections were prepared, the one was at right angle to the longitudinal axis of the thallus and the other parallel with it. For studying the calcification at the initial stages of thallus development, mature tetraspores of *Amphiroa ephedraea* were sown on the glass slide and cultured in the laboratory of the Shimoda Marine Biological Station.

Results and Discussion

The results of the quantitative analyses of the principal inorganic constituents of several calcareous algae are shown in Table 1.

Table 1. Inorganic constituents of algae belonging to Corallinaceae and Chaetangiaceae.

	Species	% of dry weed							Molecular ratio CO_3/Ca	$CaCO_3$ % of dry weed, calculated from CO_3 content
		Total ash	Calcium	Magnesium	CO_3	SO_4 Total	SO_4 Ash	FO_4		
Corallinaceae	*Amphiroa aberrans*	62.52	28.21	5.36	38.22	13.03	5.58	0.34	0.90	64.97
	A. crassissima	58.28	27.72	4.34	38.99	12.00	5.41	0.34	0.93	66.28
	A. ephedraea	61.47	29.01	4.29	40.21	11.57	5.28	0.34	0.92	68.36
	A. dilatata	60.25	28.05	5.05	38.62	12.56	5.46	0.31	0.92	65.65
	Joculator maximus	62.27	28.12	4.82	38.37	12.89	5.68	0.30	0.91	65.23
	Cheilosporum jungermannioides	60.09	28.14	5.72	38.74	12.19	5.18	0.31	0.92	65.86
	Corallina pilulifera	63.13	27.89	5.32	39.35	12.13	5.48	0.34	0.94	66.90
	Lithophollum okamurai	66.86	32.04	4.98	41.28	13.12	6.05	0.34	0.86	70.18
Chaetangiaceae	*Actinotrichia fragilis*	50.13	25.25		34.21				0.90	58.16
	Galaxaura fastigiata	56.72	25.02		35.22				0.94	59.87
	G. falcata	40.18	8.98		12.44				0.91	21.15

It can be seen that calcareous red algae contain an exceedingly large quantity of ash, amounting on a dry weight basis to 60 to 67 per cent in Corallinaceae and to 40 to 58 per cent in Chaetangiaceae. Calcium contents were found to be about 28 to 32 per cent for Corallinaceae and about 8 to 25 per cent for Chaetangiaceae respectively. These values are nearly the same as found in other species of coralline algae[1,5]). The relatively high content of magnesium, 4 to 6 per cent, seems to be characteristic

of Corallinaceae, since the magnesium content of other red algae such as *Chondrus crispus* (1.42%), *Gracilaria confervoides* (0.29%), *Delesseria sanguinea* (0.45%)[16]) as well as green algae with lime incrustation such as *Halimeda* (0.26%)[17]), was low compared with coralline algae. It is to be noted in this respect that the ratio of the content Ca/Mg of calcareous algae, 5 to 8, is reversed in the sea water, ca. 1/3[15,17]).

Table 1 also indicates that nearly all of calcium exists in form of its carbonate, a result conforming generally to the findings of earlier workers[2-5]). A slight deficit of carbon dioxide versus calcium indicates the binding of a small part of calcium with some other acids, among which ester sulfuric acid and organic acids* may be considered probable.

Further it was found that coralline algae contain a considerable amount of sulfate. When tested for free sulfate with the fronds of *Joculator maximus* and *Amphiroa dilatata* by extracting with 10 per cent hydrochloric acid at 15 to 17° and examining with barium chloride, only a negligible amount of barium sulfate was produced indicating free sulfate being nearly absent from these algae.

A comparison of the results of sulfate estimation in ash on the one hand and in acid hydrolysate on the other suggests that almost all of the sulfate exists as mono-ester sulfate, since by incineration half the amount of sulfate of this form will be lost as volatile oxide[18]). The ester sulfate may be considered to exist in part as polysaccharide sulfate with galactose as chief sugar constituent, that is found universally in red algae[18]).

The content of phosphate in coralline algae is much smaller than that of sulfate. As with sulfate the content of phosphate varies only slightly from species to species.

As early as 1882 Berthold reported that the deposition of calcium begins to take place at an early stage of the development of the algae[19]). The author has examined the course of lime incrustation with the germinating tetraspores of *Amphiroa ephedraea* as follows. Freshly harvested mature tetraspores were sown on several slide glasses which were placed in Petri-dishes containing sea-water. The dishes were kept in the laboratory at about 20° (early in June) at the side of window facing south-west. Under these conditions germinated spores grew rapidly, usually attaining 16-cell stage within 20 hours. The germlings on the slide were examined microscopically under ordinary light as well as with crossed nicols. As shown in Fig. 1, where early stages of the development are seen, the calcification occurs as early as at 2-cell stage. With the progress of the development the lime incrustation increases gradually until heavy deposition, as illustrated in Fig. 2, is reached.

The accumulation of lime starts at the middle lamella of the cell wall and gradually extends to the inner part. The incrustation of lime seems to play an important role for the maintenance of the tissure structure of coralline algae, as can be seen in the following observation. When dilute (ca. 5%) hydrochloric acid was dropped on the microscopic section of the frond of *Joculator maximus* and examined under the microscope, brisk gas evolution was observed with simultaneous dissolution of the incrusted lime. If then the section is lightly pressed under cover glass, the tissue is seen to loosen and the constituent cells become separated each other.

* As will be shown in another paper of this series comparatively large amount of organic acids is present in calcareous algae.

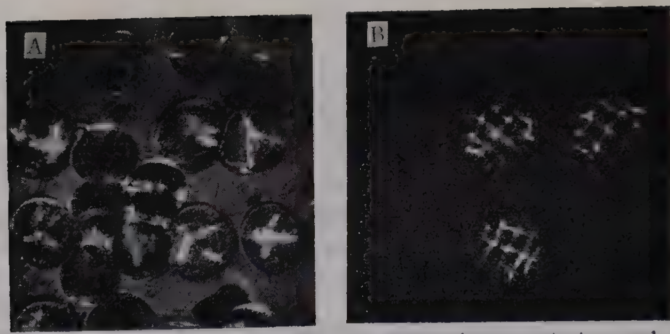


Fig. 1. Germination tetraspore of *Amphiroa ephedraea* observed under crossed nicols. Spores of various stages of cleavage are seen with lime incrusted part appearing bright ($\times 600$). A. Early stage. B. Advanced stage.

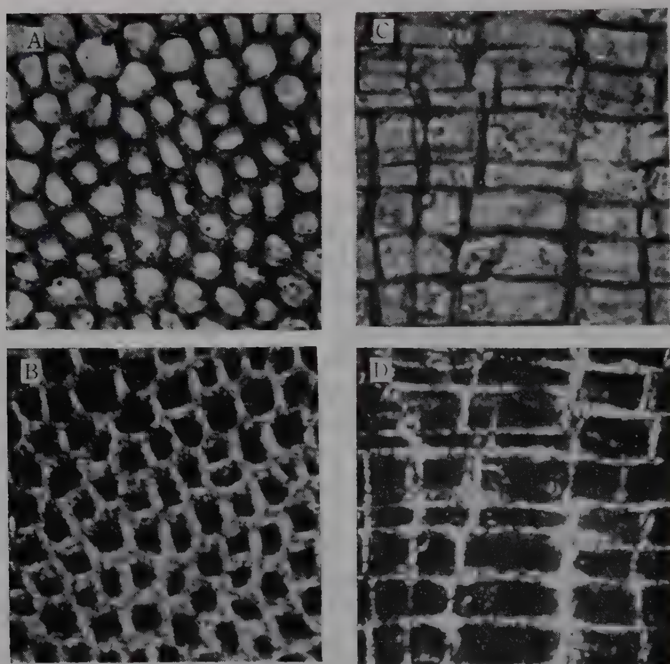


Fig. 2. Sections of mature thallus of *Joculator maximus* showing heavy incrustation of lime at the cell wall ($\times 600$). A. Section at right angle to the axis of growth, under ordinary light. B. The same under crossed nicols. C. Section parallel to the axis of growth under ordinary light. D. The same under crossed nicols.

Summary

1. With 11 species of calcareous red algae, of which 8 belonging to Corallinaceae and 3 to Chaetangiaceae, the content of Ca, Mg, CO_3, SO_4 and PO_4 was estimated.

2. Calcareous red algae were found to contain a remarkably large quantity of calcium carbonate, its amount being 65 to 70 per cent in Corallinaceae and 21 to 60 per cent in Chaetangiaceae.

3. The incrustation of lime was examined with the developing tetraspores of *Amphiroa ephedraea* and found to start as early as at the first cell division.

4. Magnesium content of coralline algae was found to be generally high compared with that of non-calcareous red algae.

5. An appreciable quantity of sulfate is present in calcareous red algae. Its content in ash is about half as much as found in the acid hydrolysate of the frond, indicating its occurrence in monoester form. The content of ionizable sulfate is exceedingly small, if existing at all.

6. Phosphate content of coralline algae is low and in nearly the same order as of non-calcareous red algae.

The author wishes to thank Prof. T. Miwa of the Tokyo University of Education for his kind guidance throughout the course of this work. Thanks are also due to Dr. M. Chihara of the Shimoda Marine Biological Station for his kindness shown in the collection of the materials as well as in the cultivation of the tetraspores.

This study was supported in part by a Grant in Aid for Development of Scientific Research of the Ministry of Education.

References

1) Haas, P., Hill, T. G., and Karsthens, E. K. H., Ann. Bot. **49**: 609 (1935). 2) Blinks, L. R., In Manual of Phycology, 263 ed. by Smith., G. M. Waltham (1951). 3) Baas-Becking, L. M., and Galliher, E. W., J. Phys. Chem. **35**: 467 (1931). 4) Haas, P., and Hill, T. G., Biochem. J. **27**: 1802 (1933). 5) Mägdefrau, K., Flora **128**: 50 (1933). 6) Grüss, J., Ber. Deutsch. Bot. Ges. **37**: 531 (1919). 7) Nomura, I., unpublished. 8) Fritsch, F. E., The structure and reproduction of the algae **2**: 409 Cambridge Univ. Press. (1945). 9) Naylor, G. L., and Russell-Wells, B., Ann. Bot. **48**: 635 (1934). 10) Russell-Wells, B., Nature, **133**: 651 (1934). 11) Dewar, E. T., and Percival, E. G. V., J. Chem. Soc. 1622 (1947). 12) Hassid, W. Z., J Amer. Chem. Soc. **55**: 4163 (1933). 13) Jones, W. G. M., and Peat, S., J. Chem. Soc. 225 (1942). 14) Roche, J., Desruisseaux, G., and Baddoin, N., Comp. rend. Soc. biol. **143**: 519 (1949). 15) Berg, R., J. prakt. Chem. **115**: 178 (1927). 16) König, J., und Bettels, J., Ztschr, für Nahr-, u. Gen-mittel **10**: 459 (1905). 17) Prat, S., and Hanachava, J., Studia, Bot. Cechica. **7**, (2/4): 112 (1946); Biol. Abstr. **24**: 5652 (1950). 18) Haas, P., and Russell-Wells, B., Biochem J. **17**: 696 (1923). 19) Berthold, G., Mitt, Zool., Stat, Neapel **3**: 393 (1882).

摘　　要

古谷庫造：石灰藻類の生化学的研究　1. 紅藻石灰藻類の無機成分について

石灰藻類の炭酸石灰沈着の機構を明らかにする目的の第一歩として，紅藻石灰藻サンゴモ科 8種，ガラガラ科 3種の主な無機成分の分析および，二，三のサンゴモ科の顕微鏡観察を行なった結果を報告する．

紅藻石灰藻はカルシウムを多量に含有し，胞子発芽直後から細胞膜間に，炭酸石灰として，活潑に沈着し，フサカニノテでは，二細胞時代にすでに炭酸石灰の沈着が見られる．藻体の炭酸石灰量は乾量に対して，サンゴモ科 65～70%，ガラガラ科 21～60% である．サンゴモ科ではマグネシウム量が他の紅藻より一般に多量である．硫酸もマグネシウムと同様に，他の紅藻よりかなり多量に存在する．灰分中の硫酸は藻体の酸加水分解によって得た全硫酸の約半量である．このことから石灰藻には，他の紅藻と同様，モノエステル型の硫酸が存在しているものと思われる．遊離型の硫酸塩はほとんど存在しない．燐酸含有量は少なく，サンゴモ科には他の紅藻にくらべて特に多い事実は認められなかった．（東京学芸大学生物学教室）

Bot. Mag. Tokyo, **73**: 360—364 (September 25, 1960)

The Microbiological Studies of the Lakes of Volcano Bandai II. Ecological Study on Aquatic Hyphomycetes in the Goshikinuma and Akanuma Lake Group

by Shizuo SUZUKI* and Hiroyoshi NIMURA**

Received April 6, 1960

Most natural fresh waters contain various kinds of fungi often designated roughly as water-molds. Not only do these molds abound in fresh waters of all sorts, but they also represent many genera and species. The aquatic Phycomycetes are the members of a dominating group among aquatic fungi, but many other species have been found in lakes and rivers.

Aquatic Fungi Imperfecti, particularly representatives of Hyphomycetes, are very common in various types of habitats such as lakes and running waters of brooks and rivers[1,2,3]. Though extensive works have been accumulated concerning the distribution and taxonomy of these fungi, no attention has been paid to the ecology of them. The writers visited the lakes of the Goshikinuma and Akanuma Group in July, 1959, for the ecological studies on aquatic Hyphomycetes.

Experimental method

Leaves fallen in the water and attacked by the fungi were brought back into the laboratory in stoppered polyethylene sacks as soon as possible. Six to eight leaves were collected in each lake at the different stations. The leaves were put in shallow petri dishes with sterilized water immersing almost all of the leaves or stems, and allowed to stand at 20°. The conidiophore and conidia were found on the submerged leaves within a day. Later examination was carried out under low microscopic power. A piece of leaf was stained with lactic phenol cotton blue to make observation in detail. The frequency of fungi in the total leaf samples was examined in each lake.

Physico-chemical specificity of lakes

The lakes studied by the writers belong to the Goshikinuma and Akanuma Group, and are situated at the foot of Volcano Bandai, Fukushima Prefecture. They were formed at the time of the famous eruption of Volcano Bandai in 1888. They have small and shallow basins. Table 1 shows the physico-chemical data of the lake waters sampled in July 1959[4]. The surface water temperature was 14–23°.

According to physico-chemical characters, the lakes were divided into the following two types:

1. Acidotrophic type (pH:3.8–5.8)
   Bishamon-numa, Midoronuma, Benten-numa, Aonuma, Rurinuma, Akanuma, Koke-numa, Hyōtan-numa, Aodoronuma
2. Harmonic type (pH:6.2–6.5)
   Yanaginuma, Jimushonuma, Nishiyanaginuma, Yarokunuma. Tatsunuma

One of the most remarkable characters of the former type is the strong acidity

* Department of Microbial Chemistry, Faculty of Pharmacy, Tokyo College of Science, Ushigome, Tokyo, Japan.

** Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.

Table 1. Physico-chemical data of the Goshikinuma and Akanuma Lake Group.

Lake types	Lakes	Water Temp. °C	pH	SO_4 mg./*l*.	Ca mg./*l*.	Cl mg./*l*.	Fe mg./*l*.	Mn mg./*l*.
Acidotrophic	Akanuma	14.0	3.8	324	166	106	14.55	1.8
	Hyotan-numa	16.5	4.1	442	133	162	0.25	1.9
	Kokenuma	16.0	4.3	442	144	202	0.16	2.6
	Aodoronuma	19.0	4.4	326	101	150	0.07	1.7
	Aonuma	17.5	4.6	260	181	116	0.04	1.7
	Rurinuma	22.0	4.7	353	195	68	0.17	2.2
	Benten-numa	23.0	5.2	364	186	148	0.01	1.7
	Bishamon-numa	21.0	5.2	429	76	128	0.18	1.4
	Midoronuma	15.0	5.8	235	134	182	0.76	1.0
Harmonic	Tatsunuma	19.0	6.2	300	95	122	0.28	0.9
	Nishiyanaginuma	18.5	6.3	154	76	106	—	0.2
	Yanaginuma	19.5	6.5	178	65	116	0.08	0.1
	Jimushonuma	19.0	6.5	153	64	112	1.75	0.2
	Yarokunuma	18.0	6.5	235	70	130	0.04	—

of the lake water, caused by the sulphuric acid from the acidic rivers of volcanoes or underground springs. The lakes belonging to this type are the inorganic acidotrophic type. The water colour of these lakes is wonderfully pale blue. On the other hand, the harmonic type reacts as neutral and the water is dark yellow green as in the water of other eutrophic lakes in general.

The water contains large amounts of mineral components and it is measured about 1–2 g./*l*.[5,6]). Especially sulphate, calcium and chlorine are present in large amounts. The amounts in the surface water of each type are as follows:

	Acidotrophic type	Harmonic type
SO_4 mg./*l*.	235–442	153–300
Ca mg./*l*.	76–195	64–95
Cl mg./*l*.	68–202	106–130
Mn mg./*l*.	1.0–2.2	0.1–0.9

It is an intresting fact that the water of the harmonic type reacts neutral in spite of containing large amounts of mineral elements.

Distribution of aquatic Hyphomycetes

The aquatic Hyphomycetes were distributed widely in the harmonic lakes as well as in the acidotrophic ones. The standing crop of aquatic Hyphomycetes, however, differed with the lake type, and it was more abundant in the harmonic lakes than in the acidotrophic ones. The fungi were found on almost all leaves sampled from the harmonic lakes, while they were very scarce in the acidotrophic ones. The result indicated that the fungus production in lakes had close correlation with the pH value of water as well as the amount of mineral elements.

The temperature of the water plays a rather important role in the production of aquatic Hyphomycetes. According to Tubaki's experiment[3]), the limiting of temperature for the growth of these fungi is about 25–27°, while the sporulation occurs only below 25°. During the writers' visits in July, 1959, the water temperature of the Goshikinuma Lake Group was 14–23°, and the aquatic fungi were found abundantly

in these lakes.

With some possible exception of certain species, the fungus flora of the lakes of Volcano Bandai seems to have close relation with the diversity in chemical properties of the lake waters (Table 2).

Table 2. Distribution of aquatic Hyphomycetes in the lakes of the Goshikinuma and Akanuma Group

Lake types	Lakes	*Anguillospora longissima*	*Articulospora tetracladia*	*Flagellospora curvula*	*Lemonniera aquatica*	*Lunulospora curvula*	*Tetrachaetum elegans*	*Tetracladium marchalianum*	*Tricladium gracile* var. *oxyphilum*	*Triscelophorus monosporus*	*Alternalia* sp.
Acidotrophic	Akanuma	−	−	−	+	−	−	−	+	−	−
	Hyotan-numa	−	−	−	−	−	−	−	++	−	−
	Kokenuma	−	−	−	−	−	−	−	+	−	−
	Aodoronuma	−	−	−	−	−	−	−	+	−	−
	Aonuma	+	−	−	−	−	−	+	+	−	−
	Rurinuma	−	−	−	−	−	−	−	+	−	+
	Benten-numa	−	−	−	−	+	−	−	−	−	−
	Bishamon-numa	+	−	−	+	−	−	−	−	−	−
	Midoronuma	+	−	−	−	−	−	−	+	−	−
Harmonic	Tatsunuma	−	−	−	−	+	++	−	−	++	−
	Nishiyanaginuma	++	−	−	−	+	+++	−	−	+	−
	Yanaginuma	−	+++	−	+++	++	++	++	−	++	−
	Jimushonuma	−	−	−	+	−	−	−	−	−	−
	Yarokunuma	+	+	+	−	−	−	−	−	−	+

Five species were obtained from the acidotrophic lakes, while nine species were found in the harmonic lakes. The group of aquatic Hyphomycetes seems generally to have a world-wide distribution. Among the fungi belonging to known species, *Tricladium gracile* var. *oxyphilum*, *Tetrachaetum elegans*, *Lunulospora curvula* and *Triscelophorus monosporus* were the most common in the lakes. However, some of the fungi require their own special biotope.

The most interesting species is *Tricladium gracile* var. *oxyphilum*. This species was found in the acidotrophic lakes, but never in the harmonic lakes. The fungus somewhat differed from *T. gracile* in either morphological or physiological natures.

Lemonniera aquatica and *Anguillospora longissima* were distributed both in the acidotrophic and the harmonic lakes. These species were found in the acidic water containing large amounts of mineral acids. On the other hand, many species were obtained from the harmonic lakes, but *Tricladium gracile* var. *oxyphilum* was not obtained from the harmonic lakes.

Physiological characters of some aquatic Hyphomycetes

So far as ths studies in the lakes of Volcano Bandai are concerned, the chemical specificity of lake waters seems to be the most essential factor. Because of physico-chemical specificity of acidotrophic lakes, the occurrence of a dominating quality of some one species was seen, and other species of aquatic Hyphomycetes were very

rare or non-existent. In order to understand the adaptability of fungi to the acidotrophic water, the writers made the following experiment.

The aquatic Hyphomycetes were inoculated in pure culture on yeast extract glucose agar, on which the conidia formation did not take place. A piece of mycelium of these fungi was submerged in the lake water which was taken from the lakes Goshikinuma Group. After this was allowed to stand for a day or two at room temperature (15–20°), the formation of conidia in the lake water was examined. The results of these experiments are given in Table 3.

Table 3. Effect of the lake waters upon the formation of conidia in some aquatic Hyphomycetes

Lake types	Lakes	*Anguillospora longissima*	*Articulospora tetracladia*	*Clavariopsis aquatica*	*Lemonniera aquatica*	*Tetrachaetum elegans*	*Tricladium gracile*
Acidotrophic	Akanuma	+++	−	−	+	−	++
	Hyotan-numa	+++	−	−	+	−	+++
	Kokenuma	+++	−	−	+	−	+++
	Aodoronuma	+++	−	−	+	−	+++
	Aonuma	+++	+	+	+	−	+++
	Rurinuma	+++	−	−	++	−	+++
	Benten-numa	+++	−	−	+	−	+++
	Bishamon-numa	+++	+	+++	++	−	+++
	Midoronuma	+++	+++	+++	+++	+++	+++
Harmonic	Tatsunuma	+++	+++	+++	+++	++	+++
	Nishiyanaginuma	+++	+++	+++	+++	+	+++
	Yanaginuma	+++	+++	+++	+++	+++	+++
	Jimushonuma	+++	+++	+++	+++	+++	+++

The different features of the formation of conidia are recognized with each species and different kinds of lake water. *Tricladium gracile* had the highest resistance against the disharmonic water. The conidia formed in the water of the acidotrophic lakes as well as in the harmonic ones. *Anguillospora longissima* is perhaps suitable to the acidotrophic lakes. The conidia formed even in the strong acidic water containing large amounts of mineral acids.

On the other hand, the formation of conidia of *Tetrachaetum elegans* and *Articulospora tetracladia* took place only in the sampled waters of the harmonic lakes and a weak acidic lake, Lake Midoronuma, but never in the other acidotrophic lakes. The experimental result of conidia formation of *Clavariopsis aquatica*, which was not obtained from the lakes of Volcano Bandai, is the same as the above mentioned two species.

The conidia of *Lemonniera aquatica* are abundantly produced in the harmonic lakes, while they are relatively scarce and slow in production in the acidotrophic lakes. The fungus seems to be suitable to the acidotrophic water more easily than do *Tetrachaetum elegans*, *Articulospora tetracladia* and *Clavariopsis aquatica*. The writers also isolated *Lemonniera aquatica* from Lake Benten-numa and Akanuma, waters of which show in testing strong acidity. These facts are in accordance with the

results in the laboratory experiments.

According to these cultural experiments, the waters of Lake Akanuma, Hyōtan-numa, Kokenuma, Aodoronuma, Rurinuma and Benten-numa are toxic to the aquatic Hyphomycetes. On the other hand, the waters of Lake Aonuma and Bishamon-numa are not so toxic to the fungi as was considered previously. These differences in the toxicity may perhaps be caused by the acidity of lake water, but not the chemical components.

Summary

The distribution of aquatic Hyphomycetes was studied in the lakes of Volcano Bandai, Fukushima Prefecture, Japan.

According to the physico-chemical characters, the lakes are divided into acidotrophic and harmonic types. The lake waters of the former type contain large amounts of mineral acids, while those of the latter contain relatively few acids.

The aquatic Hyphomycetes are more prevalent as well as better in quality in the harmonic lakes than the acidotrophic ones. *Tricladium gracile* var. *oxyphilum* is distributed only in the acidotrophic lakes, but never in the harmonic ones. *Lemonniera aquatica* and *Anguillospora longissima* seem to adapt to the acidotrophic lakes containing large amounts of mineral acids. On the other hand, *Articulospora tetracladia*, *Lunulospora curvula*, *Tetrachaetum elegans* and *Triscelophorus monosporus* are distributed only in the harmonic lakes.

The experiments were carried out in the laboratory on the resistance of the aquatic Hyphomycetes against the acidotrophic water. *Tricladium gracile*, *Lemonniera aquatica* and *Anguillospora longissima* have a high resistibility to the acidotrophic water. These results are in accordance with the observations by the writers in natural lakes.

The writers wish to express their thanks to Prof. H. Indoh and Prof. H. Ito for their instructive guidance and advice. Also to Prof. T. Tatsuno, Drs. S. Ichimura and T. Matsumoto, the writers are indebted for much valuable advice during this work.

References

1) Ingold, C. T., Trans. Brit. mycol. Soc. **25**: 339 (1942). 2) ——, ibid. **26**: 148 (1943). 3) Tubaki, K., Bull. Nat. Sci. Mus. **3**: 249 (1957). 4) Suzuki, S., Jap. Jour. Ecol. (in press). 5) Yoshimura, S., Negoro, K., and Yamamoto, S., Geogr. Rev. **12**: 10, 126 (1936). 6) ——, ——, and ——, ibid. **13**: 1147 (1937).

摘　要

鈴木静夫・二村坦孝:　磐梯山周辺の湖沼の微生物学的研究
II.　五色沼・赤沼湖群の水棲不完全菌類

裏磐梯の五色沼および赤沼湖群において，水棲不完全菌類の分布と湖水の水質との関係を観察した．湖水が中性の調和湖には水棲不完全菌類の種類が豊富であるが，多量の無機塩類を含有し強酸性を呈する酸栄養湖には *Tricladium gracile* var. *oxyphilum* が優占的で，その他に *Anguillospora longissima* と *Lemonniera aquatica* が少数見られるにすぎない．

純粋に培養した水棲不完全菌類をこれら湖群から採水した湖水中で培養したところ，上記の種は酸栄養型の湖水中でも分生子が形成されるが，調和湖だけに分布している *Tetrachaetum elegans*, *Articulospora tetracladia*, *Clavariopsis aquatica* では非調和性の強い酸栄養湖の湖水中では分生子の形成は見られない．「この事実は湖沼型によって分布する水棲不完全菌類の種類が異なることを裏づけるものといえる．(東京理科大学薬学部微生物化学教室・東京教育大学理学部植物学教室)

Bot. Mag. Tokyo, **73**: 365—368 (September 25, 1960)

A Newly Found Terrestrial Alga From Japan, *Fritschiella tuberosa* Iyengar*

by Masaru AKIYAMA** and Hiroyuki HIROSE***

Received May 25, 1960

The genus *Fritschiella* has been established by M. O. P. Iyengar[4]) in 1932. The genus was based on a single species, *F. tuberosa* Iyengar which was found in India. Later in 1939 M. S. Randhawa[6]) reported the present alga also from India. In 1941 the dioecious, isogamous reproduction and the existence of an isomorphic alternation of generations of the present species were revealed by R. N. Singh[7]). Successively A. J. Brook (1953[1]), 1956[2])) reported that the present species grew in Sudan of Egypt and had many peculiarities as a terrestrial alga. In addition to the above authors, M. O. P. Iyengar (1950)[5]) and F. E. Fritsch (1950)[3]) explained morphology and ecology of that alga in their text-books.

No report has been made of the present alga from Japan. As M. Akiyama, one of the present authors, has found recently the present alga from Shimane Prefecture, they wish to make a brief report on the present species from Japan with its habit, habitat, and its morphological characteristics that are described as follows. Before going further the authors wish to offer their thanks to Mr. S. Tanifuji of Hokkaido University who has sent them many of materials from Sapporo.

Habit. The association of the present alga can be encountered as deep green, nappy spots of macroscopic size, usually 1–5 mm. in diameter and are often associated with another terrestrial members such as *Oedocladium*, *Zygogonium* or protonema of moss-plants, mostly growing on more or less hard, but humid muddy soils, most desirably on foot-paths between rice-fields.

Diagnosis. Strata minute, 1–5 mm. high, densely ramified (Fig. 1, A, E). Stratum consists of four systems, namely, rhizoidal system, prostrate system, primary projecting system and secondary projecting system (Fig. 1, A, Fig. 2, B).

Rhizoidal system (Fig. 2, E, F) consists of uniseriate rhizoids that are issued from ends of prostrate system, sometimes branched, and cells are 3μ to 6μ broad and 40μ to 60μ long and a rhizoid mostly consists of 3 to 5 cells. Cell-size is more or less smaller than that of the type species described by Iyengar.

Prostrate system (Fig. 1, D, Fig. 2, A) consists of rosary-like filaments of 5～6 or more clusters of cells. Each cluster consists of 2～4 cells. Diagonal or perpendicular divisions of each cell of a prostrate filament lead to a cluster (Fig. 2, A). A cluster is 15μ to 25μ in diameter. Every cell is filled with chloroplasts. Number of the chloroplasts, their shape, and the existence of pyrenoids are not clear.

Primary projecting system (Fig. 1, B, Fig. 2, B, C) consists of uniseriate filaments which are branched mostly alternately and sometimes unilaterally. The component cells of the filament are almost as long as broad, 7μ to 12μ broad and 5μ to 12μ long, and each one is filled with a parietal laminate chloroplast which contains

* Dedicated to Prof. Hajime Matsuura and Prof. Yukio Yamada celebrating their sexagenery birthdays.

** Biological Institute, Faculty of Literature and Science, Shimane University, Matsue, Japan.

*** Department of Biology, Faculty of Science, Kobe University, Kobe, Japan.

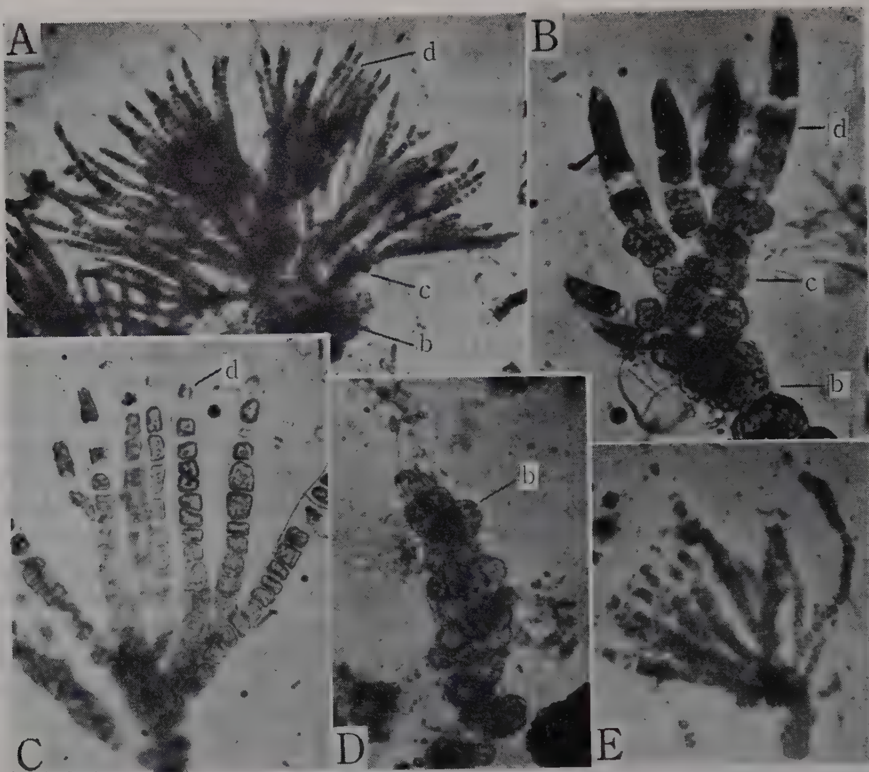


Fig. 1. A. whole view of strata ×100. B. secondary and primary projecting system ×430. C. secondary projecting system ×330. D. prostrate system ×400. E. whole view of a stratum ×100. b. prostrate system. c. primary projecting system. d. secondary projecting system.

mostly 3 or 4 pyrenoids and in some cases single pyrenoid.

Secondary projecting system (Fig. 1, C, Fig. 2, B, C, D) is an uniseriate branch that is issued from the primary projecting system and consists of an uniseriate series of cylindrical cells which are always much longer than broad and 6μ to 10μ broad and 8μ to 30μ long and containing a parietal laminate chloroplast on which 1–6, mostly 4–5 pyrenoids are present. Terminals of branches are either round (Fig. 2, D) or acutely conical (Fig. 2, C).

Growth pattern. It was found, by means of culture experiments, that the trend to form clusters within a prostrate portion appears under the dry condition, but on the contrary the occurrence of projecting filament is dominant in moist environment. These two trends are understood to be strongly related to the grade of moisture of the habitat. These trends were also actually observed on natural population of each different habitat.

Habitat. The present alga was found for the first time at Hokki, Tsuda and Shimodekisu of Matsue city of Shimane Prefecture. Later it was found that this alga grew not only in those territory along the coast line of San'in, between Tottori city and Masuda city, but also in the campus of Hokkaido University in Sapporo city of Hokkaido. The alga seems probably to be widely distributed in Japan.

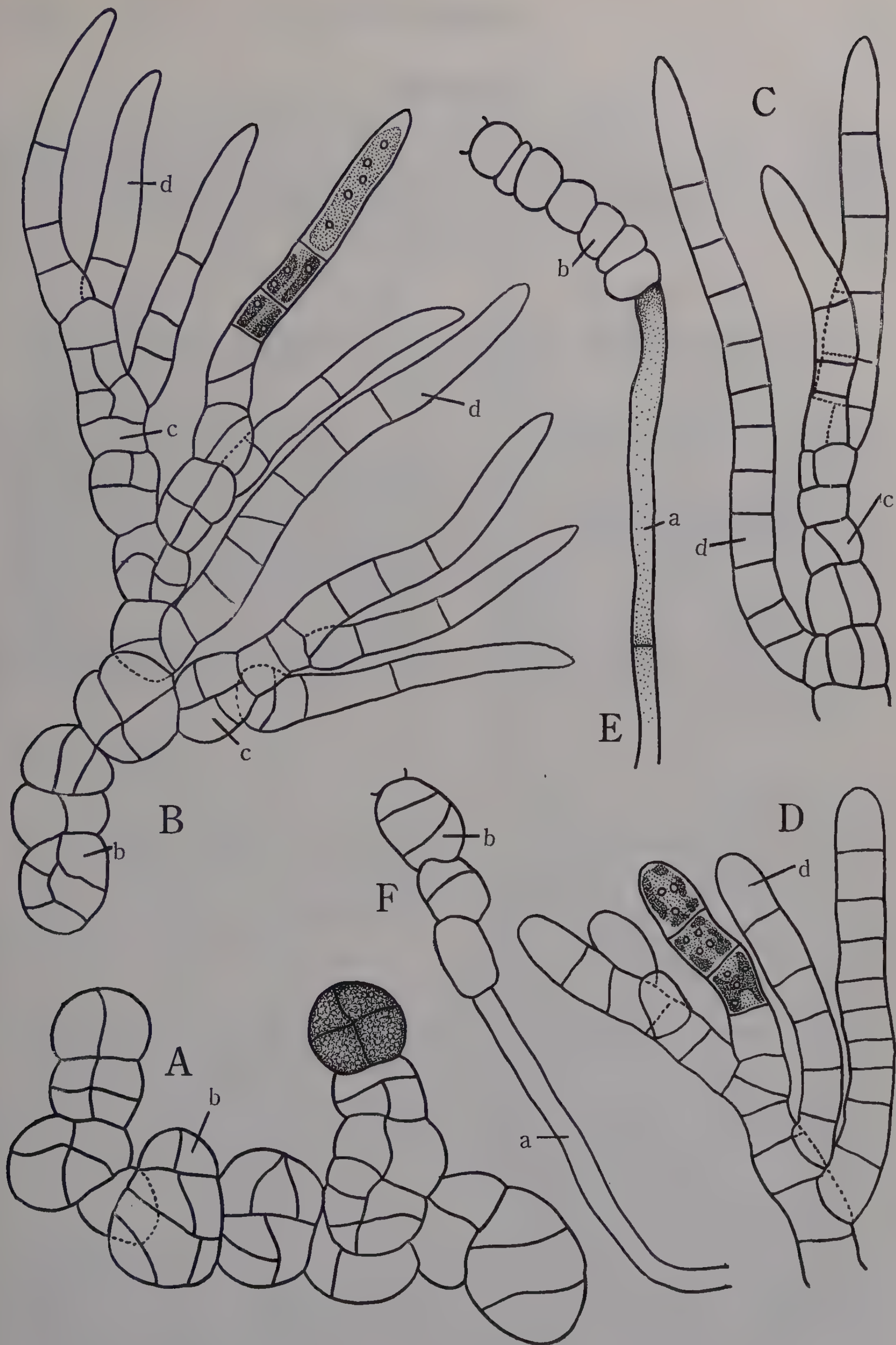


Fig. 2. A. a portion of prostrate system ×750. B. primary and secondary projecting system; cell-contents of only three cells of secondary projecting system are drawn, showing a laminate chloroplast with several pyrenoids ×750. C. upper portion of a stratum, showing a filament of primary projecting system and three filaments of secondary projecting system ×750. D. secondary projecting system whose terminal cells are rounded ×750. E. septate rhizoid ×750. F. non-septate rhizoid ×750. a. rhizoid. b. prostrate system. c. primary projecting system. d. secondary projecting system

References

1) Brook, A. J., Nature **164**: 754. (1952). 2) ——, New Phytol. **55**: 130 (1956). 3) Fritsch, F. E., Struct. and Reprod. of Algae I: 249 (1950). 4) Iyengar, M. O. P., New Phytol. **31**: 329 (1932). 5) ——, Chlorophyceae in G. M. Smith's Mannual of Phycol. : 21 (1951). 6) Randhawa, M. S., Arch. Protistenk. **92**: 131 (1939). 7) Singh, R. N., New Phytol. **40**: 170 (1941).

摘　　　要

秋山 優・広瀬弘幸：日本新産地上藻の1種 *Fritschiella tuberosa* Iyengar について

本邦新産地上藻の1種である緑藻 *Fritschiella tuberosa* Iyengar の形態的ならびに生態的な形質について記載した.

1. 本藻の産状については，主に水田のあぜ道など比較的湿潤な土壌表面に，1～5 mm 程度のひろがりをもつ不規則な円型または不整型のコロニーを形成し，しばしば，他の地上藻の *Oedocladium*, *Zygogonium* や蘚類の原糸体などと混生する.
2. 形態的には，1次，2次の直立糸，および細胞塊ならびに仮根より成るほふく糸より構成されている.
3. 生育環境とくに水分条件により，2次直立糸の成長状態が異なり，一般的に湿潤な場所に生育するものでは，2次直立糸がいちじるしく発達している.
4. 本藻の分布については，これまでに，山陰地方一帯ならびに北海道（札幌）からの産出が認められているが，本邦各地に，かなり広範に分布するものと推察する.（島根大学文理学部生物学教室・神戸大学理学部生物学教室）

Bot. Mag. Tokyo **73** : 369-376 (September 25, 1960)

ゴマの後期胚形成と組織分化

塙　　　　順*

Jun HANAWA*: Late Embryogeny and Histogenesis in *Sesamum indicum* L.

1960 年 2 月 26 日受付

種子植物の発生学においては，従来ほとんどすべての研究は胞子および配偶体の発達，胚乳形成，ならびに胚形成の初期における分割の様式などを主として扱かってきた[1,2]. これに反し胚形成の後期における組織分化を扱かった研究は少なく，被子植物については Nast[3]), Miller and Wetmore[4,5]), Reeve[6,7]), Buell[8]), 裸子植物については Allen[9,10]), Spurr[11,12]), Sterling[13]) らがあげられるにすぎない. 一方，生長点における生長と分化の問題についてはおびただしい研究がなされているが，胚における生長点の形成過程についての研究は極めて少ない. それゆえ，胚発生のすべての段階を通じての研究がなされ，そこにおいて組織系の分化と，殊に生長点の成立過程とが広く追求されることが必要であろう.

本研究ではゴマにおける胚形成後期の組織分化を述べる. これにつづく報告において. めばえ以後生殖段階までの生長点の発達が述べられる予定である. なお，ゴマの胚のう形成と胚形成のやや簡略な記述は以前に報告されている[14]).

材料と方法

開花後，種子が熟するまでの約 30 日間にわたって，蒴果から種々の発育段階にある胚珠をとりだしてフォルマリン・酢酸・アルコール混液で固定し，パラフィン切片とした. 切片は 8μ に切り，デラフィールドのヘマトキシリンで染めた.

観　　察

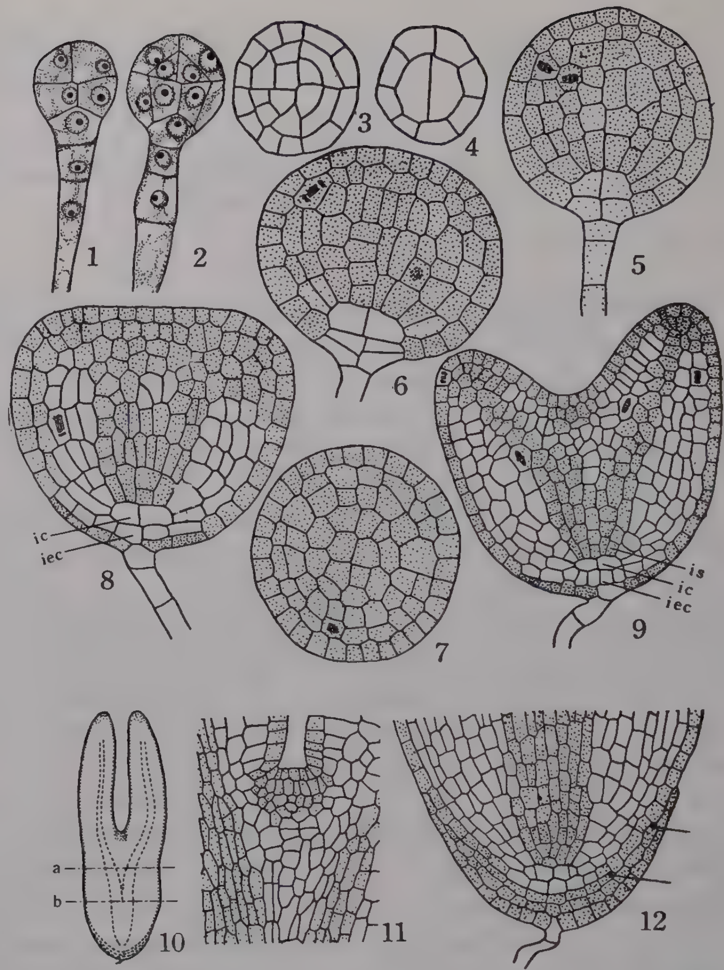
1: 初期の段階

前に報じた如く[14])，ゴマの胚形成は Onagrad 型 (Johansen, 1950)[2]) に従う. 開花後 3～4 日で 8 細胞の胚球と 3 細胞の胚柄とから成る前胚ができる (Fig. 1). 胚球の八分円体はそれぞれ周辺に平行に分裂する (Fig. 2). それによって作られた周辺細胞は，以後周辺に垂直な分裂のみを経て表皮を形成するに至ることは Fig. 2 ないし Fig. 7 によって明らかである. この表層細胞は，幼根の根冠がはじめて作り出されるとき 2，3 の細胞が周辺に平行に分裂するのを除いては，胚の全表面で周辺に垂直な分裂のみをする. 他方，八分円体の分裂によって生じた内側の細胞は周辺に平行ならびに垂直な分裂を経て(Fig. 3～7)，開花後 5 日には 5～6 層の横の細胞層と 8 列の縦の細胞列を作り，胚球は直径約 60 μ に達する (Fig. 5). この段階では各細胞は等しくよく染まる.

他方，胚柄の上端の細胞は胚球の生長に伴ないその内部にはまりこむ. この細胞はまず横に分裂し，生じた 2 娘細胞は互に直交する 2 回の縦分裂によって 8 細胞になる (Fig. 5). 胚柄の細胞と共にこれらの細胞は，胚球の他の細胞よりも明らかに染まりが弱く，また形態的には胚の軸に対して横の方向に長いことによって，その他の細胞との区別が判然としている. これら 8 細胞のうち上段の 4 細胞は根の皮層の始原細胞となり，下の 4 つは根の表皮および根冠の始原細胞としてはたらくようになることは Fig. 6 ないし Fig. 9. によって明らかである. 下の 4 細胞にはすぐに周辺平行な分裂が起る (Fig. 6). これは根冠形成の最初の現われであって，外側の細胞が根冠，内側のが根の表皮を形成するようになる. 染まりの弱い始原細胞からわかれた根冠細胞は，今度は胚の他の細胞よりもむしろ濃くそまる (Fig. 8). このようにして根の皮層始原細胞と表皮—根冠始原細胞は，表層（原初表皮）とともに胚形成の初期に確立する.

開花後 6～7 日，胚が球形から楕円形(長径約 70 μ，短径約 60 μ) に変形してくると共に，胚球の細

* 東京都立大学理学部生物学教室 Department of Biology, Faculty of Science, Tokyo Metropolitan University, Setagaya-ku, Tokyo.



Figs. 1–4. Embryos 5 days after flowering. Fig. 1. Octant-cell stage. Fig. 2. 16-cell stage of the spherical embryo. Figs. 3 and 4. Transections through the equator and the bottom, respectively, of a spherical embryo, Figs. 5∼7. Embryos 6 days after flowering. Fig. 5. The largest stage of the spherical embryo. Fig. 6. Ellipsoidal embryo. Fig. 7. Transection of a spherical embryo of the same stage as in Fig. 5. Fig. 8. 7-day embryo, showing the primary tissue differentiation and root initials. Fig. 9. 8-day embryo. is : stele initials. ic : cortex initials. iec : epidermis-root cap initials. Fig. 10. 11-day embryo. Figs. 11, 12. More detailed drawings of the apical region and the radicle end of the embryo shown in Fig. 10. Arrows indicate the stepwise addition of the root-cap layer. Figs. 1–7. ×448. Fig. 8. ×336. Figs. 9, 11 and 12. ×224. Fig. 10. ×52.

胞にはじめて分化が現われる．まず幼根の皮層始原細胞の上方に接する細胞のうちで，外側から第2および第3層の細胞のそまりが弱くなる (Fig. 6)．この変化は上方に向かってすすみ，上部では 3∼4 列のそまりのうすい細胞が現われる．はじめ外から第2の層はこの染色性の低下を示さないが (Fig. 8)，結局表層を残してその内側の4層程の細胞は空胞化する．この変化によって．胚の中軸部に依然よくそまる細胞群が残される．この空胞化した組織は基本分裂組織 (ground meristem) であって，胚軸の皮層を形成することになる．中軸に残されたよく染まる細胞群は，胚の中心柱を構成するもので，中軸前形成層 (procambial core) と呼ぶことにする. Figs. 8, 9 に示された胚では，中軸の細胞は染色性においては周囲の基本分裂組織にまさるけれども，形状においてはまだいちじるしい相異を示さない．この時期の中軸組織は基本分裂組織と前形成層との中間的な状態を示している．この中軸組織は Hanstein (1870)[15] の“原中心柱”であるが，彼の原組織説の固定的，決定論的な性格は Foster (1939)[16] 以来批判されているところであり，この言葉に代って，prodesmogen[8]), procambial core[4]), stele promeristem[7,9]) などが使われている．組織学的には単に前形成層と呼ぶことも可能と思われる．しかし，もう少しこの組織の性格をはっきりさせるために，中軸前形成層と呼ぶのが適当であろう．

中軸組織の上部中央にはまもなく染色性の減少し

た細胞が現われる (Fig. 8). これは内部基本分裂組織であって，のちに胚軸の髄を形成する.

外部と内部の基本組織の出現によって，胚の頂部に，もとのままの性質を保っている細胞群が区別される. これがのちに子葉と幼芽を生ずる細胞群である.

このようにして子葉出現前の胚には，外部および内部基本分裂組織によって，表層，中軸前形成層，および子葉一幼芽形成部域が区画され，幼根の皮層および表皮一根冠始原細胞が確定している. なお，根の中心柱始原細胞は，のちに述べるように，もう少しのちに中軸前形成層の最下端の細胞から組織される.

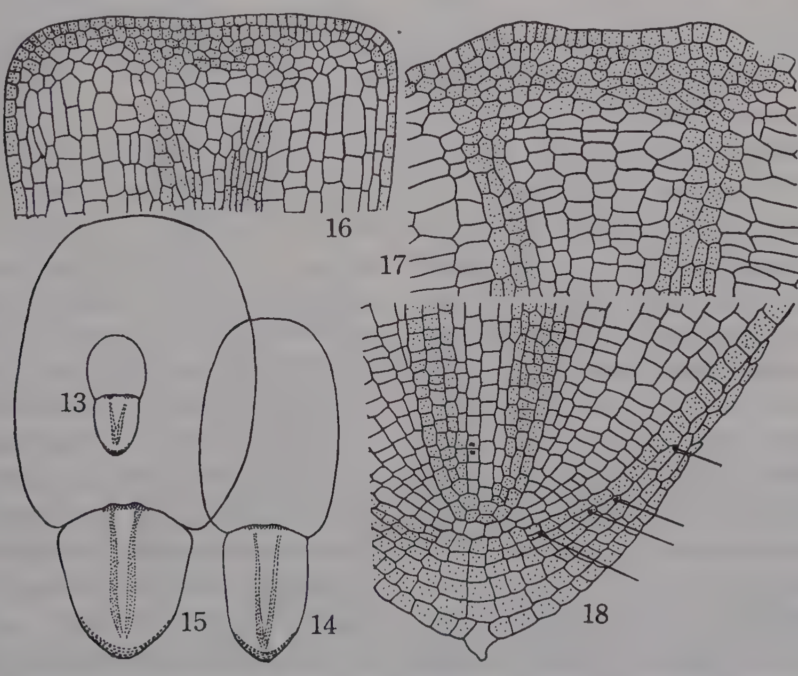
2. 子 葉

胚は開花後5日までは球形であるが，それ以後，縦の方向に短径をもつ楕円形となる，胚の上半分では縦の分裂が多く起るため，その部分が横にひろがり胚の上面が平らになる. そのため上面の両端は肩と称すべき形となる (Fig. 8). この肩の上に子葉が形成される. この肩は子葉のバットレス (buttress) である. 従って胚の上端が平らになって肩が作られたことは，子葉形成の過程が始まっていることを意味するが，この過程の始まりの時期を定めることは困難である. また一般に双子葉植物で葉の発生に際して，発生位置の第2層以下の 1〜2 層に見られる周辺平行な分裂は，今の場合認められない.

子葉原基の頂端では分裂活性が続いて，それによって頂端分裂組織が構成される. 子葉の伸長とともに，その中軸には，頂端分裂組織と胚軸の前形成層とをつなぐ濃くそまる前形成層が伸びる. これによって胚軸と子葉の維管束系ははじめから一体のものとして出現する. これは，のちに述べる維管束移行にとって重要である. 子葉の幅の増大に際しては，普通葉におけると同じく葉縁分裂組織がつくられる. そこには周辺に平行と垂直な分裂を交互にくりかえす1個の次周縁始原細胞が見られる.

3. 幼 根

開花後8日以後，子葉の伸長が進むと共に胚軸一



Figs. 13–18.
Fig. 13. 11-day embryo. Fig. 14. 14-day embryo. Fig. 15. 20-day embryo. Fig. 16. Apical region of the embryo of Fig. 13. Fig. 17. Shoot apex of a 19-day embryo. Fig. 18. Radicle tip of the embryo of Fig. 14. Arrows indicate the stepwise addition of the root-cap layer. Figs. 13–15. ×23. Figs. 16–18. ×224.

幼根はその細胞の若干の縦分裂によって太さをまし，同時に中介生長によって伸びる．皮層には顕著なリブメリステム (rib-meristem) が現われる．中軸前形成層は伸長しつつ胚の中心柱へと分化する．その先端の数個の細胞が根の中心柱始原細胞となる．それは縦断切片で見ると，最初は縦にやや長いが (Figs. 8, 9)，のちには等径的になる(Fig. 18).

皮層始原細胞は 8 日ごろまで分裂しないでもとのままの形を保っている (Figs. 8, 9). そののち分裂して横方向に細胞をわかつ (Fig. 12). しかし，胚形成を通じて皮層始原細胞は識別しやすい形を保っている．

表皮—根冠始原細胞は，始めは 4 細胞より成るが，周辺に垂直な分裂によって表皮細胞を生み出すようになると，始原細胞の数は多くなり，縦断面において 6 箇ほど認められる．この始原細胞の最初の分裂は周辺平行に起こり，外側に根冠細胞をわかつ (Fig. 6). 周辺平行な分裂はこの始原細胞に接する表層細胞の若干のものにも起こる (Fig. 8). 始原細胞は，以後しばらく周辺に垂直にのみ分裂して根の表皮を作り出す．ついで再び周辺平行な分裂が根冠細胞をわかつ．その結果，根冠の細胞層の増加は階段状である (Figs. 12, 18, 矢印).

幼根の頂端分裂組織はこのようにして割合に早く組織され，中心柱始原細胞，皮層始原細胞および表皮—根冠始原細胞を中心として明らかな組織帯の構成を示す (Figs. 9, 12). しかし，これら始原細胞群においては胚の生長を通じて分裂は少なく，幼根が胚の伸長に寄与する割合は小さい．胚の主軸の伸長量の多くの割合は，胚軸部の中介生長に負う．幼根の組織と胚軸のそれとの間には明らかな区別はないが，幼根が頂端生長によって伸長した量は，胚のうの中で剝落せずに残っている根冠の先端から上縁部までの高さに相当する．その割合は胚軸—幼根の長さの約 1/4 になる (Figs. 10, 13, 14, 15).

4．茎　頂

茎頂分裂組織の発現は胚の上端に子葉—幼芽形成部域として，もとのままの胚的な細胞群が空胞化をこうむらずに残ることによってはじまる．最初この細胞群から子葉原基がわかたれる．子葉原基が生長をはじめるとその基部に，胚軸の内部基本分裂組織(髄)につづいた染まりのうすいリブメリステムが現われ，子葉原基の伸長と共に求頂的に拡張される．このリブメリステムの出現によって，子葉の間のへこんだところに依然として染色性を保つ細胞の一群が残される (Fig. 9). これは表層をも含めてる 3 層ほどの深さをもつ．これらの細胞は胚の中でも最も分化していない細胞であって，もとの胚球の細胞が最後に残ったものと見なすことができる．Souèges (1934)[17] は子葉の間にあって，そこから茎頂が生じてくる細胞群を epiphysis と呼んで双子葉類の胚に共通なものであるとした．今の場合，子葉原基の間にある，染色性を保っている細胞群がそれである．これはまだ組織帯への分化のないことと，葉原基を作っていないことにおいて茎頂とは異なるものであるという見方もあるが[18]，現研究では，子葉発現前の子葉—幼芽形成部域を茎頂と見なす故に，"epiphysis" を頂端分裂組織の一発達段階と見なす．このことについては後に論ずる．

この細胞群はまだ明白な層状構造を示さないが，表層 (tunica) の 1 層は常に保たれていて，そこでは周辺に垂直な分裂しか行なわれない．第 1 葉原基の出現する前にこの分裂組織は子葉の間の，幅 18 μ，長さ 110μ 程の広さを占め，4〜5 層の厚さを持つようになる (Figs. 11, 16). 子葉を通る正中縦断面で見ると，この分裂組織は，周囲の空胞化した細胞によって胚軸の前形成層から遮断されているが (Fig. 11)，子葉に直角な断面で見ると，割合によく染まる 2 本の索によってそれと接続されている (Fig. 16). この索は空胞化をこうむらずに残された細胞のつながりである．第 1 葉原基の形成がはじまると，この索を通って第 1 葉葉跡として前形成層が下方の胚軸の前形成層から求頂的に分化する．

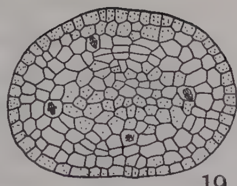
第 1 葉原基の位置は 2 本の索（前形成層）のほぼ上方にあたる．第 1 葉のバットレスが隆起してくる前，その出現位置の第 2 層または第 3 層の細胞に周辺平行な分裂は見られない．ゴマにおいては，第 2 葉以後については一般に双子葉類でそうであるように，葉の発生の兆候は，発生位置の表層下の第 2〜3 層に起った周辺平行な分裂によって明らかである．ところが第 1 葉の発現に際してはそのような顕著な分裂は見られない (Figs. 16, 17). ただし，第 2 層に周辺平行な分裂が皆無なのではなく，しばしば起る．しかしそれは第 1 葉形成に関係したものとは思われないものである．

第 1 葉原基は 11〜20 日の胚において徐々に隆起

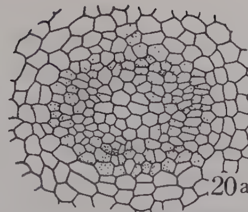
するが，胚の発達の最後の段階にあってもバットレスの状態にある (Figs. 15, 17). 生長を終えた胚においても，茎頂はまだ明白な層状構造を示さないで，いわば未完成にとどまっている．胚形成の終りの時期に，胚軸の皮層および髄の細胞がいちじるしく横に伸びる．そのため2本の第1葉葉跡を構成している細胞は，皮層や髄の細胞から，染色性のみならず形においても明らかに識別されるようになる (Fig. 17).

5. 胚中心柱

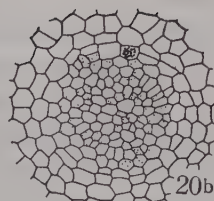
胚中心柱は前述のごとく，はじめ中軸前形成層として現われる (Figs. 8, 19). 胚軸一幼根の長さが約 300 μ に達すると，中軸前形成層の上部中心に空胞化した部分(髄)が発達して，円筒形の前形成層となる (Fig. 20a). この時期には，それは一様な円筒に見える．中軸組織の下半部では中心に空胞化が起らないで一様な中実の円柱である (Fig. 20b). Fig 20a と b はそれぞれ Fig. 10a と b に相当する断面である．胚の生長は 10 日ごろから急増するが，胚軸の伸長は大部分を中介生長に負うている．胚中心柱の伸長も同じである．この伸長の過程で前形成層の中に原生木部極と原生篩部極とが決定される．円筒の部分では4箇の原生篩部極と2箇の原生木部極の出現によって，前形成層は円筒から6角形に変わる (Fig. 21a). 原生木部は子葉を通る面内で前形



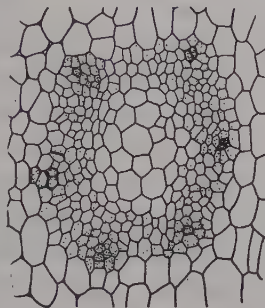
19



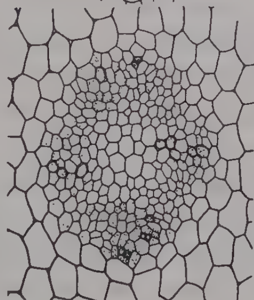
20a



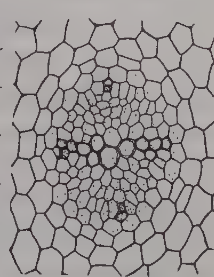
20b



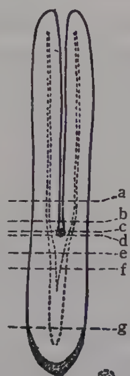
21a



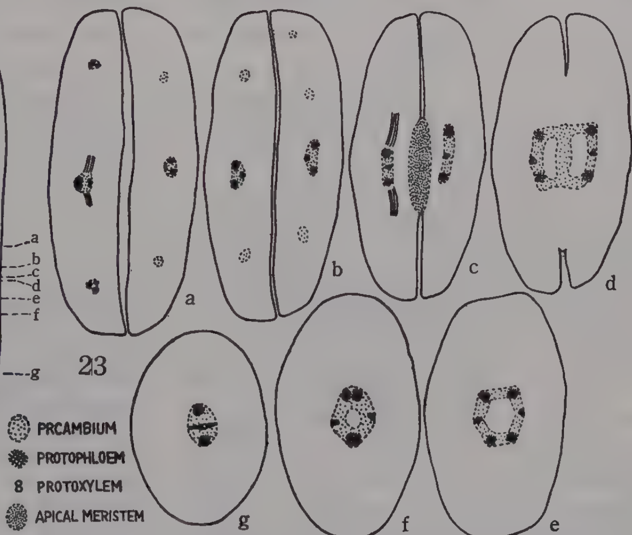
21b



21c



22



23

Fig. 19. Transection of a 7-day embryo, showing the procambial core. Fig. 20a, b. Transections of a 11-dry embryo, corresponding to the levels a and b in Fig. 10, showing the procambial cylinder and the procambial core respectively. Fig. 21 a-c. Transections of a 14-day embryo, cut at the levels e-g in Fig. 22, showing different positions of the protoxylem and protophloem poles according to different levels along the hypocotyl-radicle axis. Fig. 22. Longitudinal section of a 14-day embryo. Fig. 23 a-g. Transections of a 14-day embryo, corresponding to the levels a-g in Fig. 22, showing the vascular transition between the cotyledons and the root. Figs. 19-21. ×224. Fig. 22. ×23. Fig. 23. ×52.

成層円筒の両側に生ずる．原生篩部は他の4つの角に位置する．原生木部の細胞は，現研究での処理条件の下では，原形質の収縮を起しやすく，それによって早く識別される．原生篩部極の細胞も，木部よりやや程度は低いが同様に原形質の収縮を示し，また縦分裂によって前形成層の他の細胞よりも直径が小さいことで見分けられる．連続切片によって胚軸を下にたどれば，原生篩部極は2個ずつ互いに接近し，終に合一する (Fig. 21b, c)．幼根部では原生木部の内側に後生木部の細胞が同様に原形質収縮を示し，幼根では胚軸におけるよりも早く後生木部が現われることが知られる．Fig. 21a, b, c はそれぞれ Fig. 23e, f, g に同じものである．Fig. 23 において，連続切片を上方にたどると，幼根においては合一した2つの篩部極は上方に向かってはわかれて別の子葉に入っていくことが示される．そして幼根にあっては相対する極に位置した2つの原生篩部が，子葉の中に入ってからは次第に接近して，子葉基部よりわずか上方で終に合一する (Fig. 23d, c, b, a)．この過程で木部は向軸側に，篩部は背軸側に位置するようになり，ここに並立維管束となる．このようにして，放射型から並立型への維管束型の転換は，胚形成の途上，幼根―胚軸―子葉の維管束系の中で行なわれる．Figs. 22, 23 は14日の胚からの切片を示したもので，維管束内の木部と篩部は未熟であるが，移行部の基本的な構造は決定されている．

論　　議

1．残余分裂組織

胚における最初の組織分化は，空胞化した基本組織が局所的に現われることによって，もとのままの性質を保った細胞群が区画されることである．Meyer (1958)[19] はリンゴの胚でそのような細胞群を残余分裂組織 (residual meristem) と呼んだ．この言葉は Kaplan (1937)[20] が茎の生長点において，もとの分裂組織的性質を保っているよく染まる組織を記載するのに用いたもの (Restmeristem) である．胚組織の分化においてこれを使う場合は多少含蓄は異なるかも知れない．しかし胚における最初の組織分化の特色は，もとのままの，いわゆる胚的 (embryonal) な細胞が特殊化をこうむらずに残って行くことにあるのであるから，それらの細胞群に対して残余分裂組織という呼び名を使うことは，説明に有効であり便利でもある．すなわち，中軸前形成層，および子葉―幼芽原基はそれぞれの位置に残された残余分裂組織である．それらはそれぞれの組織へ分化して行くが，最後に幼芽原基 (epiphysis) が未分化のまま残る．これは胚における最後の残余分裂組織である．

2．表　層

ゴマの胚では，八分円体の周辺平行な分裂によって外側に作られた細胞層が“原表皮”となる．以後この表層は根冠形成の際に，ごく一部の基部の細胞が周辺平行に分裂するのを除いては，胚形成を通じて必らず周辺に垂直に分裂する．ところが *Phlox*[4]) や *Pisum*[7]) では胚の表層は，初期には周辺平行な分裂をも行なう．特に子葉や茎頂の発現に際してそれが起る．それ故これらの植物では表皮の成立はゴマにおけるよりもおそい．Reeve[7]) は，球形の胚の表層細胞は単に原初表皮 (protoderm) の前身であって，原初表皮はもっと後になって周辺平行分裂によって成立するものと考えている．ゴマの胚形成の型である Onagrad 型の発生をする胚ではおそらく表皮の成立はゴマと同じであると思われる．この他の発生型の胚でも原表皮 (Dermatogen) が記載されている[1])．しかしこれが果してそのまま表皮へ発展するかどうかについて徹底的にはしらべられていない．表皮の成立には種々の型があると考えられる．

3．幼　根

幼根の分裂組織は胚形成の割合に早い時期に，子葉の出現と同じころに，構成される．そして中心柱，皮層および表皮―根冠の3始原細胞群は，その出現のはじめから明瞭に識別される．これら始原細胞の分裂能力について，Clowes[21,22,23]) は，いわゆる始原細胞は，ほとんどあるいは全く分裂しないで静止域 (quiescent center) をなしているといっている．しかしまた彼[24])は *Sinapis* の胚において，根端の細胞はすべて活性であって，静止状態は種子の発芽中に現われてくることを示した．生長した根における静止域の存在についてはこれを支持する研究[25])も，また否定する研究[26])もある．ゴマではそれについてはまだ研究されていないが，胚の幼根においては *Sinapis* の胚と同じく細胞はすべて活性である．

4．茎　頂

Pisum[7]) や *Pseudotsuga*[9]) では茎頂の形成は子

葉の出現よりも早く，表層細胞の周辺平行分裂によってはじめられるが，ゴマではそのような明らかな兆候がないから，茎頂発現を定義することが難かしい．一方 Spurr[11]) は子葉と幼芽とを生ずる胚上端部を茎頂として解釈する．それは，子葉は'葉'であり，茎頂の第一義的な属性の一つは葉を形成する能力である，という見解に基づく．ゴマにおいても，胚の上端部を最も若い茎頂と見なし，子葉—幼芽形成部域を，胚に現われた最初の頂端分裂組織と見なす方が便利である．したがって茎頂は胚の上に創始されるのではなくて，最初に"未熟な"頂端分裂組織があって，それが発育を通じて一定の構造をもつように発展する，と解釈される．したがって，epiphysis (Souèges, 1934)[17]) は頂端分裂組織の一発達段階と見なされる．Epiphysis は胚細胞の分化の面から見れば，前述のように，胚における最後の残余分裂組織である．

ここまでの段階は，茎頂発達の第一期であって，子葉の形成はこの幼ない茎頂において行なわれる．次の段階で，充分に構成された茎頂への発展が行なわれるが，ゴマでは胚形成の間にはこの過程は完成されないで，発芽後数日たってはじめて完全な層状構造を示すようになる．この茎頂発達の第2段階で第1葉が形成される．

茎頂と胚軸の前形成層との維管束系による連絡については *Juglans*[3]), *Phlox*[4,5]) などの胚で記載されているが，その接続の最初の状態はよく見られてはいない．ゴマで観察されたところでは，頂端分裂組織が子葉基部の空胞化した細胞によって隔離されたように見えたとき，子葉と直角な面内においては，残余分裂組織の2本の索によって胚軸の前形成層と連絡されている．第1葉の形成と共に，この索は求頂的に前形成層化し，胚軸と頂端分裂組織との維管束による連絡が作られる．

5. 胚中心柱

胚軸における中心柱形成の重要な点は，維管束の移行部 (transition region) の成立にある．ここにおいて，茎部と根部とが接続されているのであるが，その移行組織の基本的配列は，その形成の最初の時期に決定される．すなわち中軸前形成層が維管束化する際，横断面に見られる原生木部と原生篩部の分化する位置は，胚軸における横断面のレベルに応じてきまっている．中心柱の下端，幼根の分裂組織と接するレベルでは2原型の放射維管束が現われるが，上方に進むにしたがって木部と篩部の位置が移動して，胚軸上部では2木部極と4篩部極とを含む筒状の前形成層が見られる．更に子葉の中へ進むと，背軸側に篩部，向軸側に木部をもつ並立維管束が現われる．このような維管束型の移行が，中介生長をする胚軸で行なわれるためには，そのような順次に変化した配列が，その分化のはじまりから胚軸のレベルに応じて決定されていなければならない．子葉と幼根は頂端生長をしつつ，それぞれ，茎部の特徴である並立維管束と，根の特徴である放射維管束とを形成する．これに対し胚軸は両者の間にあって，上端に近い程茎的であり，下端に近い程根の特徴に近い維管束系を形成する．それ故，上下両端からの，相反する影響が胚軸において作用し合って，この移行組織が決定される，という印象をうける．

文　　献

1) Maheshwari, P., An Introduction to the Embryology of Angiosperms (1950). 2) Johansen, D. A., Plant Embryology (1950). 3) Nast, C. G., Lilloa **6**: 163 (1941). 4) Miller, H. A., and Wetmore, R. H., Amer. Jour. Bot. **32**: 588 (1945). 5) ——, and ——, ibid. **32**: 628 (1945). 6) Reeve, R.M., ibid. **35**: 65 (1948). 7) ——, ibid. **35**: 591 (1948). 8) Buell, K.M., ibid. **39**: 194 (1952). Allen, G. S., ibid. **34**: 73 (1947). 10) ——, ibid. **34**: 204 (1947). 11) Spurr, A.R., ibid. 9) **36**: 629 (1949). 12) ——, ibid. **37**: 185 (1950). 13) Sterling, C., ibid. **36**: 184 (1949). 14) Hanawa, J., Bot. Mag. Tokyo **66**: 98 (1953). 15) Hanstein, J., Bot. Abhandl. Bonn **1**: 1 (1870). 16) Foster, A. S., Bot. Rev. **5**: 454 (1939). 17) Souèges, R., Bull. Soc. bot. Fr. 81: 769 (1934). 18) Philipson, W. R., Biol. Rev. **24**: 21 ((1949). 19 Meyer, C. F., Amer. Jour. Bot. **45**: 341 (1958). 20) Kaplan, R., Planta **27**: 224 (1937). 21) Clowes, F. A. L., New Phytol. **53**: 108 (1954). 22) ——, ibid. **55**: 29 (1956). 23) ——, Biol. Rev. **34**: 501 (1959). 24) ——, New Phytol. **57**: 85 (1958). 25) Jensen, W. A., and Kavaljian, L. G., Amer. Jour. Bot. **45**: 365 (1958). 26) Shimabuku, K., Bot. Mag. Tokyo **73**: 22 (1960).

Summary

1) Late embryogeny and histogenesis in *Sesamum indicum* L. were described.

2) The protoderm of the embryo is established early by the periclinal division of the cells of the 8-celled embryo-globe: the cells of the surface layer produced by this division are divided only anticlinally thereafter, except for a few cells participating in the root-cap formation.

3) The tissue differentiation in the embryo starts with the appearance of cells with decreased stainability. The first such cells appear in the outer ground meristem, which makes the embryonic cortex, then follows the appearance of such cells in the inner ground meristem, which makes the pith in the hypocotyl. In consequence of the appearance of these less-staining regions, tissues whose cells are dark-staining and appear unaltered are blocked out as a procambial core and a presumptive region of cotyledons and plumule. The procambial core differentiates into the embryonic stele.

4) The presumptive region of cotyledons and plumule is regarded as an incipient, juvenile shoot apex. The shoot apex develops from the juvenile to the advanced structure through embryogeny. Even in the full-grown embryo, however, the apical meristem remains incompletely developed. No periclinal division occurs in the 2nd or 3rd cell layer at the leaf position prior to the formation of the first foliage leaves. This makes a contrast with the initiation of later leaves, which is preceded by periclinal divisions in the 2nd and 3rd layers. The apical meristem is connected with the procambium of the hypocotyl by two meristematic strands at opposite sides of the procambial cylinder in the intercotyledonary plane before the first leaf primordia are initiated.

5) The apical meristem of the radicle is organized at about the same stage with the cotyledons, earlier than the formation of the plumule. Initials of the root cortex and those of the epidermis and root-cap are distinguished from other cells, from the time of their very origin. The undermost cells of the procambial core, which are contiguous with the cortex initials, function as the initials of the root stele.

6) The embryonic stele is differentiated as a structure having the transitional pattern of vascular system between the cotyledons and the root: its fundamental tissue pattern is already determined when the first protoxylem and protophloem poles appear.

Bot. Mag. Tokyo, **73**: 377 (September 25, 1960)

Short Communication

Atsushi TAKIMOTO*, Yoshio TASHIMA** and Shun-ichiro IMAMURA*:

Effect of Temperature on Flower Initiation of *Pharbitis Nil* Cultured *in Vitro*

滝本敦*・田島良男**・今村駿一郎*: アサガオの花芽形成におよぼす温度の影響

Received June 27, 1960

Tashima and Imamura reported previously that *Pharbitis Nil* cultured aseptically on a medium containing sucrose initiated flower primordia in total darkness. They came to the conclusion that the essential process leading to the formation of the flowering stimulus proceeded in the absence of light[1]). In their experiment, however, flower initiation occurred at relatively low temperatures of 10–15° but not at 25–29°, This was attributed to the fact that the plants were exhausted, especially severely at high temperature, by etiolation, but no further attention was paid to the influence of temperature on flower initiation.

Seeds of *Pharbitis Nil*, strain Violet, sterilized with calcium hypochlorite, were sown in 18 mm. diameter test tubes on modified White's medium containing 5% sucrose and 0.75% agar. They were then subjected to three light conditions at 10°, 1) continuous light of ca. 3000 lux from fluorescent lamps, 2) short day consisting of an 8-hour period of light from fluorescent lamps and a 16-hour dark period, and 3) complete darkness. Three months after the start of the experiment, all dissected plants have produced not only axillary flower buds but also terminal flower buds, indicating that they had received strong flowering induction irrespective of the light condition to which the plants were exposed. In the comparable experiment at 20°, no plants under continuous illumination but all under short day condition initiated flower primordia. Plants cultured in total darkness at 20° were extremely etiolated, and flower buds were observed only on 15% of them.

Under continuous illumination the difference in flowering response due to different temperature is very obvious. At low temperature the plants seem to be insensitive to the inhibiting effect of light, which at high temperature suppresses flowering entirely.

Apart from temperature, sugar added to the culture medium seems to have a pronounced effect, as our preliminary experiment indicates. Whether the strong elongation of hypocotyl caused by etiolation, which is more striking at high temperature, has some influence on the flowering response or not, remains also to be investigated.

Reference

1) Tashima, Y., and Imamura, S., Proc. Japan Acad., **29**: 581 (1953).

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan. 京都大学農学部応用植物学研究室

** Laboratory of Forestry, Department of Agriculture, Kagoshima University, Kagoshima, Japan. 鹿児島大学農学部林学教室

本　会　記　事

住 所 変 更

(35 年 1 月より 7 月末日まで)

(北海道支部)

花房　尚史　札幌市北八条西 5 丁目　北大低温科学研究所

斎藤　雄一　札幌市北九条西 9 丁目　北大農造林

(東 北 支 部)

清水(征矢野)芳孝　仙台市川内　東北大川内分校

結城　嘉美　山形市神明町 1760

田中　清　福島市浜田町 84　福島大学芸生物

吉岡　邦二　東北大理生物

柳沢　勉　水戸市堀町新田 93217

(関 東 支 部)

渡辺　良象　東京都北多摩郡保谷町上保谷大門1720

石倉　成行　東京教育大理植物

別所　礼子　小金井市東町 4-97-4

向坂　道治　練馬区上石神井 2-814

古瀬　義　栃木県栃木市皆川城川町 1864

村上　毅　杉並区高円寺 2 丁目　農林省蚕糸試験所栽桑部

宮田　渡　長野県北安曇郡白馬村　白馬高校

代谷　康　大田区 3-1-2879　粟原方

神保　忠男　豊島区目白町 4-41

有賀　裕勝　東大理植

保泉　仁子　横浜市神奈川区六角橋町　市立六角橋中学

杉野　孝雄　静岡県庵原郡由比町　由比中学

岡安　広治　長野県岡谷市西堀　岡谷東高校

斎藤　賢道　練馬区小竹町 2799　斎藤進方

小野　幹雄　都立大理生物

新関　宏夫　埼玉県鴻巣市　関東東山農業試験所

若林　裕　千葉県八日市場市イ-1630 県立匝瑳高校

松原　益太　練馬区南町 2-3686

原沢伊世夫　小金井市貫井北町　東京学芸大農学

武井　尚　川崎市菅 5091

鈴木　昌友　同上

勝見　允行　三鷹市　国際キリスト教大学生物

(北陸支部)

齊田　鋼　金沢市栄町 36

(中 部 支 部)

日野　精一　名古屋市瑞穂区田辺通 3　名古屋市大生物

牛山　六男　岐阜市南長森東中島　森崎方

楠　正貫　名古屋市昭和区天白町虹ケ丘南住宅 10 の 304・名古屋市千種区楠元町　愛知学院大学

佐藤　徳次　愛知県一宮市栄町 2-20

加茂　昌子　山梨県中巨摩郡櫛形町小笠原 255

飯島　敬造　静岡県沼津市小諏訪 180

土井田幸郎　三島市谷田　国立遺伝研

近藤　静代　愛知県豊田市寺部町 3-73

(近 畿 支 部)

村田　源　京都市右京区川島権田町 34-9

渡辺光太郎　京大農

今堀　宏三　阪大教養生物

岸　敏夫　和歌山市古屋 400　県立箕島高校

(中国・四国支部)

井木　長治　岡山県(倉敷局区内)美和町 1139

稲葉　通一　広島大理植

高橋　節　高知県須崎市新荘　須崎高校新荘分教場

丸山　巌　島根県仁多郡横田町　県立横田高校

岡村　信夫　高知市中秦泉寺 25-5

(九 州 支 部)

谷口　勇　鹿児島県始良郡加治木町　始良教育事務所

久保　淳　福岡県糸島郡志摩村松隈 767

大城　肇　九大農水産植物

田村　博美　鹿児島県出水市武本　県立出水高校

金子賢一郎　福岡学芸大久留米分校・生物

(外　国)

香村　真徳　沖縄那覇市　琉球大学文理生物

Bot. Mag. Tokyo, **73**: 379—388 (October 25, 1960)

Histochemical Studies on Embryogenesis of *Pinus thunbergii* Parl.

by Akio TAKAO*

Received March 30, 1960

In gymnosperms although there have been many studies on female gametogenesis and proembryo formation, the study on later stage of embryogenesis, especially the stage after the suspensor has elongated into the middle part of prothallium, is seldom. Spurr[1]) describes in detail on this stage of embryogenesis in *Pinus strobus*. In the present paper, the writer wishes to report the mode of embryo formation in *Pinus thunbergii*.

Hitherto the majority of investigations of embryogenesis in both angiosperms and gymnosperms were concerned mainly on the morphological point of view. The writer studied the embryogenesis by the histochemical methods and has found some interesting facts on the appearance and the disappearance of proteins and polysaccharides in the embryo as well as in the prothallial tissue surrounding embryo during the development of embryo. The result reveals the localization of these substances in the different stages of embryo.

Material and Methods

The cones of *Pinus thunbergii* pollinated in the previous year were collected from the field of the Faculty of Science, Nagoya University, during the period from the end of June to the end of October in 1956 to 1958.

The ovules were taken out of the cones, and only the prothallia were dissected out by removing the integuments from ovules. The prothallia were fixed by the following fixatives: Telyesniczky's, Bouin's, ethanol-$HgCl_2$ (50% ethanol 100 m*l*., glacial acetic acid 10 m*l*. and mercuric chloride 8 g.), 10% formalin, and ethanol-formalin (absolute ethanol 90 m*l*., formalin 10 m*l*., glacial acetic acid several drops). The fixed prothallia were imbedded in paraffin by customary methods. Sections were cut at 10 μ thick. For staining, Heidenhain's iron-hematoxylin, toluidine blue, methyl green-pyronin, ninhydrine-Schiff reaction[2]), arginine reaction[3]), Millon's reaction[4]), Lillie's polysaccharide reaction[5]) and iodine-potassium iodide (I_2–KI) solution were employed.

Results

(A) Mode of embryo formation.

The course of embryogenesis of *Pinus thunbergii* can be divided approximately into following six stages:

(1) *Proembryo.* The nucleus of a fertilized egg gives rise to four free nuclei by two successive mitoses at the center of the egg cell (Fig. 1). They migrate toward the bottom of the egg cell, but no wall is laid there (Fig. 2). Then this four-nucleated proembryo is divided anticlinally by newly formed walls. Further, a 16–

* Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan.

celled proembryo is formed by successive divisions. This 16–celled proembryo is composed of four-tiers, each tier comprising four cells (Fig. 3). There is no wall between the cytoplasm of the egg cell and the most proximal tier of the proembryo. Of these four-tiers, the most distal tier has been called the embryo tier, the next one the suspensor tier, and the third the rosette tier.

(2) *Elongation of suspensor.* The embryo tier is pushed out toward the center of prothallium by elongation of the suspensor tier (Fig. 4). As the suspensor elongates, the prothallial cells lying in front of it are broken down to make a cavity into which the suspensor penetrates. When the suspensor reaches its full length, the four cells of embryo tier at the top of suspensor give rise to the embryo initial.

In Pinaceae, polyembryony has been well known to occur not as an unusual phenomenon. In *Pinus thunbergii*, an ovule has two, three or sometimes more egg cells, and from each egg cell one set of the suspensor grows out, hence, an ovule has two or more sets of suspensors. Usually, one embryo initial is formed at the top of one set of the suspensor. Only one embryo, however, develops completely and the other embryos usually degenerate in an early stage of embryogenesis.

(3) *Early stage of embryo formation.* When the cavity has extended to about three quarters of the whole length of prothallium, the embryo initial continues vigorous cell divisions in this cavity. Thus, an embryo which is uniformly composed of rectangular cells is formed (Fig. 5).

(4) *Differentiation into two portions; the basal and the distal portions.* The distinction of two parts becomes recognizable in the embryo by appearance of a layer of flat cells between the above-mentioned rectangular cells and the suspensor cells (Fig. 6). In this paper, the region consisting of rectangular cells is called the 'distal portion' and that composed of flat cells the 'basal portion' for convenience.

In the suspensor the cells close to the embryo also divide frequently to give rise to the secondary suspensor containing somewhat round cells. Then the basal portion of embryo continues cell divisions, making about ten layers of cells (Fig. 7).

(5) *Differentiation in the basal portion.* The cells of basal portion continue to divide transversely producing flat cells. Flat cells in the periphery of this region are arranged obliquely. Thus the basal portion not only elongates toward the basal end but also differentiates into two parts, the central and the peripheral parts (Fig. 8). After this stage further morphological differentiation is scarcely seen in the basal portion (cf. Figs. 8, 9, 10 and 11).

(6) *Differentiation in the distal portion.* When the differentiation in the basal portion is occurring simultaneously the distal portion is pushed out toward the distal end of the prothallium and assumes a conical form resulting from vigorous cell divisions in the central regions of it (Fig. 8).

After the basal portion has ceased to differentiate, at the margin of the conical region four or five shoulder-like protrusions (cotyledon initials) emerge and elongate beyond the top level of the cone (shoot apex) (Fig. 10). Simultaneously the basal part of the cone conspicuously elongates with vigorous cell divisions. The growth of these portions results from the differentiation of the distal portion into three parts; i.e., the cotyledons, the conspicuously elongating axis (hypocotyl), and the cone-like shoot apex which remains in the original form. As a result of further elongation of the cotyledons and the hypocotyl, not only the basal portion is driven toward the basal end of the prothallium but also the distal portion attains to the distal end of the embryo cavity (Fig. 11). In the hypocotyl the differentiation into cortex, procambium and pith is performed.

(B) Staining of proteins

The cytoplasm surrounding four free nuclei of the proembryo is stained homogeneously with Heidenhain's iron-hematoxylin or with various staining reactions for proteins (Fig. 1). After migrating toward the egg bottom, the cytoplasm surrounding nuclei is still stained intensely with iron-hematoxylin or with protein-staining reactions, while the bulk of the egg cytoplasm is stained only faintly. In spite of absence of boundary wall between the egg cell and the four-nucleated proembryo, the cytoplasm shows different stainability between them (Fig. 2). With the formation of the eight-celled proembryo, the stainability of cytoplasm in the proembryo becomes weaker (Fig. 3).

The cells of prothallium from central cell stage to proembryo stage are highly vacuolated, and the cytoplasm of them is faintly stained with iron-hematoxylin or with protein-staining reactions. These cells may contain only small quantity of substances.

Shortly before the suspensor begins to elongate, the prothallial cells near the proembryo become rich in relatively dense cytoplasm (Fig. 2). With the elongation of suspensor, the stainability of this region becomes rapidly intenser (Fig. 4), and reaches the maximum state up to the time when the suspensor has elongated to half of its full length. Then the stainability decreases and finally disappears just after the embryo initial has been formed (Fig. 12).

On the other hand, small quantities of granules appear in the cells of the peripheral region of prothallium at the beginning of the elongation of suspensor. These granules are stained in red by ninhydrine-Schiff staining for proteins (in general), in orange red by arginine reaction or in black by iron-hematoxylin. With development of the embryo, these granules gradually increase their amounts and this tendency extends from the basal part of prothallium to the distal and also from the peripheral part to the central (Figs. 12, 13 and 14). The amount and stainability of them reach the maximum after the cotyledons have elongated (Fig. 15). In this stage, all the cells of prothallium are full of numerous protein granules (Fig. 16). This condition continues till the maturation of seed.

In the embryo, the nuclei are strongly stained and the cytoplasm is somewhat weakly stained during the whole course of embryogenesis. When the cotyledons begin to differentiate, small protein granules appear in the cells of cotyledons and in the cells behind the shoot apex. With the elongation of cotyledon these granules increase their amounts and become visible in all the cells of embryo.

(C) Staining of polysaccharides.

Before the proembryo stage, there is no granule containing polysaccharides in the cytoplasm of prothallial cells neighboring the bottom of the egg cell (Fig. 17). Granules come into sight in the prothallial part where the suspensor is to elongate (Fig. 18). These granules are considered to be starch grains, for they are positive to I_2–KI test and are stained in red with Lillie's polysaccharide staining. Concurrently with elongation of the suspensor, not only the quantity of starch grains per cell increases but also starch grains become to be contained in an increasing numer of cells (Fig. 19). This increase of starch continues up to the time when the embryo begins to differentiate into two portions. In this stage the grains come about in the cells at the distal end of prothallium, and their quantity attains to the maximum state (Fig. 20). The tissue where very many starch grains are present looks so uniformly red (with Lillie's staining) or black (with I_2–KI) that each grain is not distinguisha-

ble. Thereafter, the more the prothallial cells containing many starch grains are broken down, the larger the embryo cavity expands longitudinally and radially (Figs. 21 and 22). However, in the layers of cells surrounding the cavity small quantities of remaining starch grains and amorphous polysaccharides are seen up to the time when the cotyledons begin to elongate (Fig. 21), and thereafter disappear (Fig. 22).

In the cells of the basal part of prothallium a small number of small starch grains appear after the embryo initial begins to develop. With development of the proembryo these areas containing starch grains extend from the basal portion of prothallium to the distal, though starch grains in each cell scarcely increase in their amounts. This condition is kept up to the stage of differentiation of cotyledons. Thereafter, in the cytoplasm of all the prothallial cells, an amorphous pale red stain with Lillie's staining is seen besides starch grains, hence in the stage after the differentiation of cotyledons the prothallial cells contain amorphous polysaccharides as well as small starch grains.

In the proembryo, a small quantity of amorphous polysaccharides but no starch grain exists in the cytoplasm and the nuclei. In suspensor cells, somewhat intense stains of polysaccharides but not of starch grains are observed. In the cells of embryo, starch grains begin to appear from the time of differentiation into two portions. At first small quantities of starch grains appear in the cells of the basal portion of embryo and increases with the development of embryo. With further differentiation of the basal portion, the grains increase in their amounts and become to be distributed in the central part and the periphery (Fig. 21). At last many starch grains as well as much of amorphous polysaccharides are seen throughout the basal portion of embryo but not in the distal portion (Fig. 22).

(D) Nucleic acid staining.

In the four-nucleated stage of proembryo, the cytoplasm surrounding the free nuclei is stained in pale purplish blue with toluidine blue or in faint pink with methyl green-pyronin. After the nuclei have migrated to the bottom of the egg cell, the cytoplasm is strongly stained with above-mentioned dyes.

In the cells of embryo the nuclei are stained in blue with toluidine blue or bluish purple with methyl green-pyronin, and the cytoplasm is in reddish purple with toluidine blue or pink with methyl green-pyronin. But any change in the stainability with these dyes is not seen during all the stages of embryogenesis.

In the cells of prothallium, the nuclei are well stained with above-mentioned dyes throughout all stages. On the other hand, the cytoplasm is only faintly stained with these dyes in the stages before the elongation of suspensor, but with appearance of protein granules and starch grains in the prothallial cells these stainabilities become detectable and are held out throughout stages after the suspensor has elongated.

Discussion

In a proembryo, it is a prominent phenomenon that cytoplasm surrounding the nuclei intensely stained with various protein-staining reactions and with Heidenhain's iron-hematoxylin. The stainability of this cytoplasmic area reaches the maximum in four-nucleated stage and disappears in eight-celled stage. Shimamura[6]) already confirmed that the strong basophilic stainability of this area results from the presence of RNA. It is probable from these facts that the substances in these area are mainly proteins and RNA, and are utilized for proembryo formation.

In prothallium of *Pinus thunbergii* the cells surrounding the cavity in which embryo develops contain numerous starch grains as well as abundant protein granules. Straus *et al.*[7]) elucidated in maize endosperm cultured *in vitro* that starch was a considerably good carbon source next to sucrose, fructose, glucose and maltose, and in higher concentrations (4 or 8%) starch stimulates the growth. Also in isolated endosperm of papaw, starch was found to be useful as a carbon source for growth (Lampton[8])). Furthermore, Straus[9]) observed in morphological studies on maize endosperm that the starch-accumulating cells are gradually broken down and absorbed by younger and more active cells. These investigations suggest that the starch is useful also *in vivo* for the growth of tissue. Straus *et al.*[7]) reported that the maize endosperm in tissue culture required yeast extract as a nitrogen source for growth. Thus, in the early stage of embryogenesis of *P. thunbergii* it is also probable that the growth of suspensor and embryo is performed at the expense of proteins supplied from the neighboring tissues. It seems that starch and proteins are converted into substances of low molecular weights with breakdown of the prothallial cells and these nutrients are transferred from prothallium to embryo through the embryo cavity. In angiosperms, the probability of transportation of the nutrients from nucellus to embryo through the embryo-sac cytoplasm was suggested in leguminous plants (Takao[10,11])).

At the time when above-mentioned substances are absorbed by the embryo it is interesting that at first the proteins rapidly disappear and somewhat later the starch gradually disappears though a small quantity of them is left afterwards. A faster disappearance of proteins than that of starch does not seem to result from the quantity of them, but rather it may be actively converted into substances of low molecular weights to utilize for the embryo formation.

Konar[12]) found that in the gametophyte (prothallium) of *Pinus roxburghii* several layers of cells surrounding the embryo are devoid of any visible reserve material. In *P. thunbergii*, on the contrary, small quantities of starch grains and amorphous polysaccharides remain in the cells of two or more layers surrounding the embryo cavity after the embryo has started to differentiate. However, the most part of this remaining starch and other polysaccharides disappear in the mature stage of seed.

There have appeared many studies on starch in the endosperm of angiosperms (Bernstein[13]), Duvick[14])). The problem whether the embryonic cells contain starch has been discussed. For instance, Netolizky[15]) in Caryophyllaceae and Buell[16]) in *Dianthus* observed on starch in the embryo, whereas Devine[17]) reported that there are many starch grains in the cortex and the cotyledon of mature embryo of *Lychinus*. In the embryo of *Pinus thunbergii*, small quantities of starch grains appear first in the cells of the peripheral region close to suspensor. Then accumulation of much of amorphous polysaccharides as well as of many starch grains is begun in the basal portion of embryo, but nearly no accumulation is seen in the distal portion. On the other hand, there are only small quantities of small starch grains and of amorphous polysaccharides in the cells of prothallium. These substances, therefore, may be too little to serve as reserve materials. On the contrary, in *P. roxburghii* of Konar[18]) many starch grains in the gametophyte (prothallium) may remain till the maturation of seed. The presence and absence of starch in the embryo and endosperm is probable to depend on difference of species.

Duvick[14]) reported in the endosperm of maize that some of the small cytoplasmic granules give a positive reaction to Millon's reagent, they are stained yellow with

iodine and they are not birefringent under the polarization microscope. He concluded that these granules consist, for the most part, of protein, and he called them "protein granules". In *P. thunbergii* though at first restricted in the basal part of the prothallium, these protein granules rapidly increase in their amounts and at the maximum condition they are contained in all the cells of prothallium. It seems that these protein granules are the reserve material and are analogous to those of maize endosperm.

Konar[18]) reports in *Pinus roxburghii*, that alcohol-soluble nitrogenous substances in the embryo and gametophyte (prothallium) decrease and are converted into proteins after differentiation of the cotyledon initials. In *P. thunbergii* small protein granules appear in the embryo from the stage of elongation of the cotyledon, and they are considered to be the storage material for the coming germination. Except for a little difference in the timings, Konar's survey[18]) is in agreement with the present morphological and histochemical studies in *P. thunbergii*.

The mode of differentiation of *Pinus thunbergii* is scarcely different from that of *P. strobus* which Spurr[1]) described.

Summary

On the mode of embryogenesis in *Pinus thunbergii*, its course can be divided approximately into six stages.

With histochemical methods the appearance and disappearance of proteins and polysaccharides are studied. The localization of these substances in the different stages of embryogenesis is elucidated.

In the cells of the middle part of prothallium where the suspensor elongates, abundant starch and proteins begin to appear in the proembryo stage. These substances rapidly increase and reach the maximum state in the case of proteins when the suspensor has elongated to half of its full length, or in the case of starch when the embryo begins to differentiate into two portions. These substances disappear when the cells containing them break down. The proteins completely disappear just after the embryo initial has formed, and after some intervals the most of starch gradually decreases its amount till the basal portion of embryo begins to differentiate into two parts though a small quantity of them remains in the tissue surrounding the embryo. From the above facts it may be concluded that these substances are converted into soluble state and serve as nutrients for the early development of embryo.

Protein granules begin to accumulate in the remaining prothallium from the stage of suspensor elongation and in the embryo from the stage of cotyledon differentiation. These protein granules gradually increase their amounts and in the mature stage of embryo attain to large quantities. Also many starch grains and much of amorphous polysaccharides begin to accumulate in the basal portion of embryo from the stage of cotyledon differentiation. These substances are considered as the storage material for the coming germination.

The author expresses his deep sense of gratitude to Prof. Dr. T. Shimamura for his kind guidance, and also his grateful thanks to Mr. T. Ôta for his helpful advice and criticism.

References

1) Spurr, A. R., Amer. J. Bot. **36**: 629 (1949). 2) Yasuma, A., and Ichikawa, T., J. Lab. Clin. Med. **41**: 296 (1953). 3) Serra, J. A., Stain Technol. **21**: 5 (1946). 4) Pearse, A. G. E., Histochemistry. Theoretical and Applied. (London) (1954). 5) Lillie, R. D., Stain Technol. **26**: 123 (1951). 6) Shimamura, T., Bot. Mag. Tokyo **69**: 524 (1956). 7) Straus, J., and LaRue, C. D., Amer. J. Bot. **41**: 687 (1954). 8) Lampton, R. K., Thesus. Univ. Michigan Ann. Arbor. (1952). 9) Straus, J., Amer. J. Bot. **41**: 833 (1954). 10) Takao, A., 23rd annual meeting of Bot. Soc. of Japan. (1958). 11) ——, 24th annual meeting of Bot. Soc. of Japan. (1959). 12) Konar, R. N., Phytomorphology **8**: 168 (1958 a). 13) Bernstein, L., Amer. J. Bot. **30**: 517 (1943). 14) Duvick, D. N., ibid. **42**: 717 (1955). 15) Netolizky, F., Anatomie der Angiospermen-Samen. In Linsbauer's Handbuch der Pflanzenanatomie. Abt. II, Teil 2, Bd. X (Berlin) (1954). 16) Buell, K. M., Amer. J. Bot. **39**: 458 (1952). 17) Devine, V., ibid. **37**: 197 (1950). 18) Konar, R. N., Phytomorphology **8**: 174 (1958 b).

摘　　要

高尾昭夫:　クロマツの胚発生の組織化学的研究

クロマツの胚発生の様式をしらべ，6 時期にわけて記述した．更に組織化学的方法を用いてでんぷん，その他の多糖類およびたんぱく質の消長を胚発生のいろいろな時期についてしらべた．

胚柄がのびだしていく時，その前面にある前葉体細胞は，受精前はほとんどどんな物質も検出されないが，前胚形成が始まるとでんぷん粒とたんぱく質が現われ，それらの量は急激に増加する．たんぱく質は胚柄が完成時の半分の長さにのびた頃，でんぷん粒は少し遅れて胚が 2 部分に分化する頃に最大量に達する．これらの物質を含む細胞は崩れて前葉体内に腔所をつくり，この腔所内で胚発生がすすむ．その後たんぱく質は急激に消失し胚原基ができると完全になくなる．でんぷん粒の大部分は胚の基部が 2 部分に分化する時までに徐々に消失し，少量のでんぷん粒と無定形の多糖類が腔所の囲りに残る．これらの物質は胚発生の初期に水溶性の物質に変えられて胚の生長と分化に使われるものと考えられる．

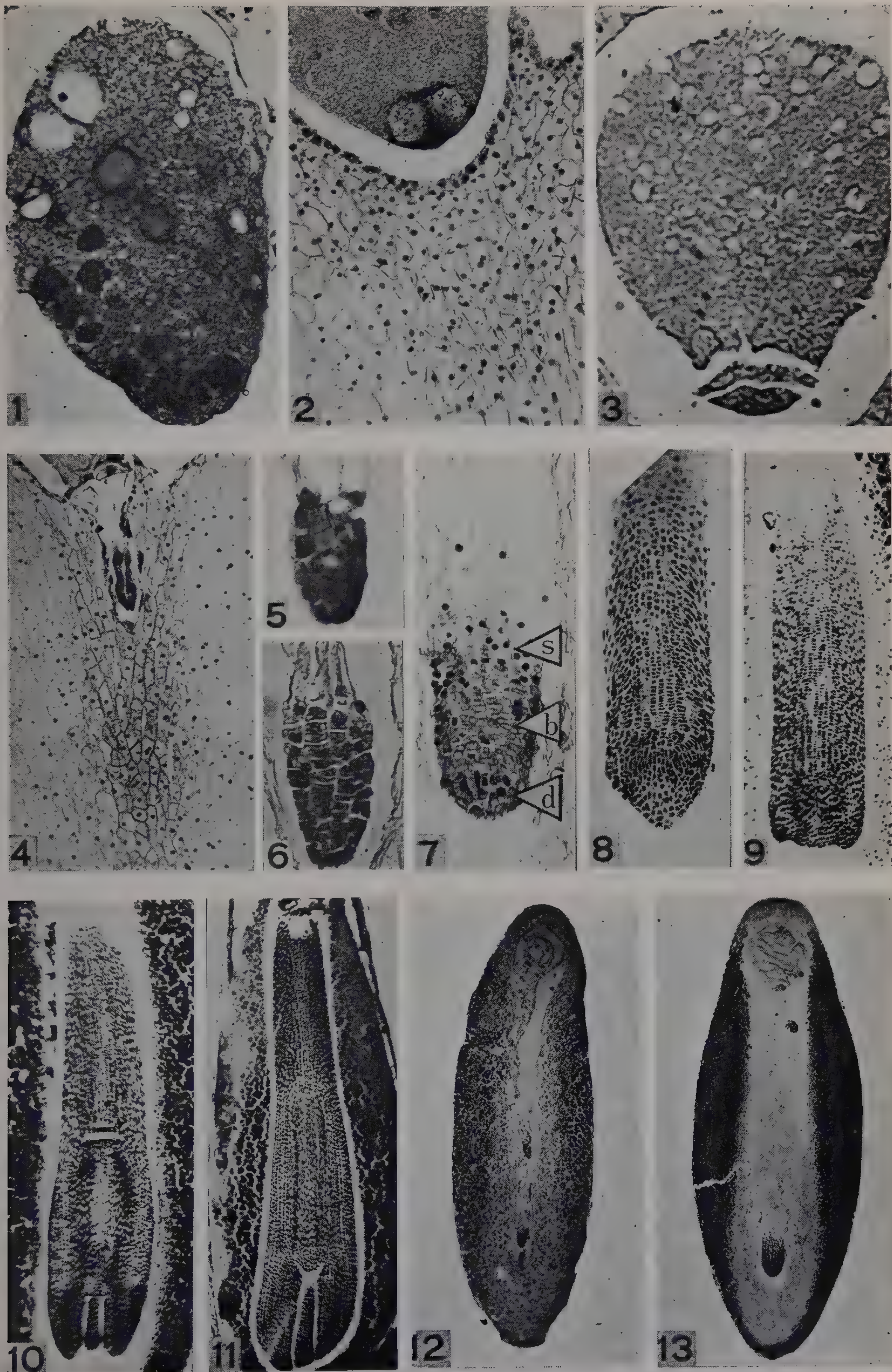
胚柄がのびだす頃から前葉体周辺部の基部の方の細胞にたんぱく粒が現われ，胚発生がすすむにつれて前葉体の中心部および先端部の細胞にも現われその量も増加する．この増加は種子の成熟まで続き，成熟時には胚をとり囲む残余の前葉体細胞に多量のたんぱく粒が貯えられる．

胚では 2 部分に分化する頃，基部にでんぷん粒が現われ，基部の分化生長につれて量を増し，更に多量の無定形の多糖類も現われ，種子の成熟時には胚の基部全体にこれらの物質がみられる．更に子葉が分化しはじめる時から小さいたんぱく粒が現われ成熟時には胚全体の細胞にみられるようになる．これらはすべて発芽の時の貯蔵物質と考える．(名古屋大学理学部生物学教室)

Explanation of Figures

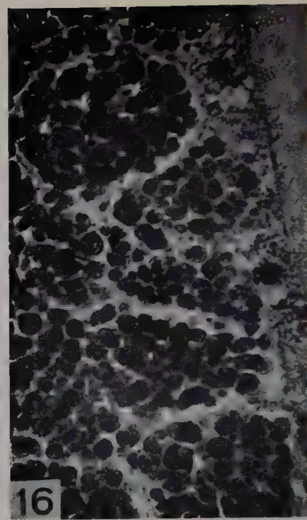
Fig. 1. Four nucleated proembryo stained with ninhydrine-Schiff reaction (only two of the four free nuclei are seen). Cytoplasm surrounding the nuclei is stained homogeneously. ca. 140×. Figs. 2–11. Development of proembryo and embryo. Stained with Heidenhain's iron-hematoxylin. Fig. 2. Four-nucleated proembryo at the bottom of egg cell (two nuclei are seen). Cytoplasm of proembryo is stained intensely, and the prothallial cells neighboring the egg bottom become rich in relatively dense cytoplasm. ca. 140×. Fig. 3. 12-celled proembryo composed of three tiers. The stainability of cytoplasm in the proembryo becomes weaker. ca. 140×. Fig. 4. Beginning of the elongation of suspensor. The prothallial cells lying in front of suspensor is stained intensely. ca. 70×. Fig 5. Embryo in early stage. The embryo is composed of rectangular cells similar in shape to each other. ca. 160×. Fig. 6. Beginning of the differentiation into two portions. The distal portion is composed a few layers of rectangular cells and the other portion is of flat cells. ca. 120×. Fig. 7. Later stage than Fig. 6. The distal portion (d) composed of rectangular cells, the basal portion (b) containing flat cells and the secondary suspensor (s) composed of somewhat round cells are seen. ca. 90×. Fig. 8. Differentiation in the basal portion. The central part is composed of transverse flat cells and the peripheral part is of oblique cells. The distal portion assumes a conical form. ca. 40×. Fig. 9. Shoulder-like protrusions (cotyledon initials) at the margin of conical region (shoot apex). ca. 40×. Fig. 10. Cotyledon initials elongate, and the part behind the shoot apex also elongates. ca. 30×. Fig. 11. The distal portion is seen elongating to the distal end and the basal portion reaches the basal end of the prothallium. ca. 18×. Figs. 12–13. Prothallium as a whole. Stained with iron-hematoxylin. Fig. 12. Prothallium in the stage similar to Fig. 6. The cells of peripheral region of the prothallium contain protein granules, but the inner region surrounding the embryo cavity is free of them. ca. 18×. Fig. 13. The stage similar to Fig. 7. The area containing protein granules extends to the distal end of prothallium. ca. 18×.

Figs. 14–15. Prothallium as a whole. Stained with ninhydrine-Schiff reaction. Fig. 14. The stage similar to Fig. 10. The area containing protein granules extends throughout the whole prothallium. ca. 14×. Fig. 15. Prothallium and embryo in mature stage. All cells of remaining prothallium are full of protein granules. ca. 15×. Fig. 16. A part of prothallium in the stage similar to Fig. 15. In each cell many protein granules are seen. Stained with ninhydrine-Schiff reaction. ca. 600×. Figs. 17–22. Changes in polysaccharides in the course of embryogenesis. Lillie's polysaccharide staining. Fig. 17. The stage just after fertilization. The contents of prothallial cells are not stained. ca. 110×. Fig. 18. Eight-celled proembryo at the egg bottom. Large quantities of starch grains are seen in the prothallial cells a little apart from the egg bottom. ca. 120×. Fig. 19. Beginning of elongation of the suspensor. The cells lying in front of suspensor are rich in starch grains. ca. 110×. Fig. 20. Embryo and prothallium in the stage similar to Fig. 6. Starch grains in the cells in front of the embryo cavity reach the maximum in their amounts. ca. 110×. Fig. 21. Prothallium and embryo in the stage similar to Fig. 10. Small quantities of starch grains and of amorphous polysaccharides remain in the cells surrounding the embryo cavity. In the embryo, starch is contained in the central region and the periphery of the basal portion. ca. 14×. Fig. 22. Prothallium and embryo in mature stage. The layer surrounding the embryo cavity contains small quantities of starch grains. The basal portion of embryo has large quantities of starch grains and amorphous polysaccharides. ca. 14×.

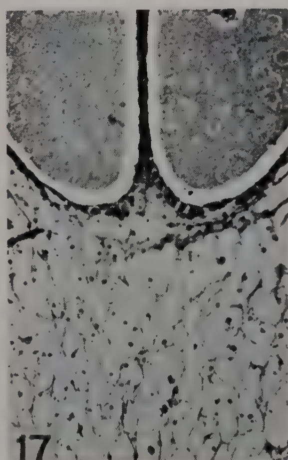

1
2
3
4
5
6
7
s
b
d
8
9
10
11
12
13


14

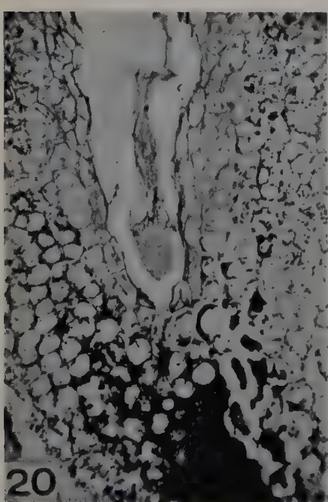

15


16


18


17


19


20


21


22

Bot. Mag. Tokyo, **73**: 389—397 (October 25, 1960)

Effect of Water Economy on Plant Growth 3. Effect of Partial Excision of Root System on the Dry Matter Production of Sunflower Plant*

by Tsumugu TOTSUKA**, Tetsuo OSHIMA***, and Masami MONSI**

Received January 8, 1960

Fukumoto and Takahashi[1]) observed the depression of growth induced by cutting of roots at wheat cultivation. Recently, Humphries[2]) has reported that difference of final yield was induced by partial removal of root system in barley plants cultured with solutions of varying mineral salt concentrations.

It may be sure that partial root excision affects the growth of plant indirectly as well as directly; the direct effect was brought about by injury of root system and by the consequent depression of mineral nutrient absorption as mentioned by Humphries[2]), and the indirect one, by the change in physiological processes accompanied with limited water uptake through the reduced root system.

Here, it will be discussed on the basis of the results obtained in previous studies[3,4]) that the growth depression of sunflower plants with partially excised root systems was induced by the decline of net assimilation with transient unbalance in water economy of leaves. The influence of partial root excision on a plant growth must be of a very importance to discuss the effect on a subsequent growth of injury of subterranean parts by strong wind or by transplanting and weeding, especially in crop plants, many problems remaining to be studied in future.

Material and Method

The experiment was performed in the experimental field of the Tokyo University of Education at Hoya, Tokyo, in the summer of 1958. As material, *Helianthus annuus* L., variety " Large Russian ", was used because of its high sensitivity to wilting and of its horizontally wide-spread root system, as illustrated by Weaver[5]), appropriate for the partial root excision. After soaking in tap water for about 3 hours, the seeds of nearly equal weight were sown on June 3 in a regular disposition with 80 cm. spacing, which was decided to eliminate the mutual shading of plants throughout the experimental period on the basis of the data of Clements *et al.*[6]). The soils, a kind of Kanto loam, being fertilized previously with compost and slaked lime, have shown the permanent wilting point of 25 per cent on a dry weight basis and the field capacity of 63 per cent.

Thirty-nine days after sowing, the plants grew in average about 43 cm. in height, 100 g. in fresh weight, and their lateral roots elongated almost horizontally 26 cm. in maximum length, and the tap root reached vertically 21 cm. depth. A half number of the plants were excised their lateral roots by cutting the rhizosphere in a circle of 10 cm. diameter up to 20 cm. depth around the plant with a semi-cylindrical aluminium plate (10 cm. in chord). The eliminated part was 28 per cent in total dry weight of

* Supported by the Grant in Aid of Scientific Research of the Ministry of Education.

** Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.
*** Sanjô Junior High School, Mizuhashi-machi, Toyama Pref., Japan.

the root system, or 61 per cent in fresh weight of the lateral roots (ref. Table 1). The control plants and the treated ones were respectively designated as the A- and B-sets.

Table 1. Effect of a partial root excision on the growth in leaf area (sq.cm./plant), root system (g.f.w./plant, and g.d.w./plant) and active root/leaf area ratio ($=C_w/\bar{F}$ ratio, mg.f.w./sq.cm.).

			Control plant (the A-set)				Treated plant (the B-set)			
			Jul.12	Jul.19	Jul.26	Aug.2	Jul.12	Jul.19	Jul.26	Aug.2
Leaf area			1323	3105	5340	9300	1323	2267	4160	7100
Root	lateral	f.w.	5.6	14.8	25.0	51.2	2.2	14.6	22.3	38.7
		d.w.	0.26	1.32	2.15	3.0	0.10	1.57	1.75	3.6
	tap	f.w.	1.1	4.9	21.2	43.7	1.2	3.0	17.3	35.7
		d.w.	0.24	0.38	1.65	3.4	0.26	0.23	1.35	3.0
$C_w/\bar{F}$ ratio			4.2	4.7	4.7	5.5	1.6	6.4	5.3	5.4

The sampling was carried out at daytime between 10 a.m. and noon, three times at constant intervals of a week, starting from July 12 (the same day of root excision). Fresh and dry weights of leaves, stems, roots and flowers, and leaf area were measured at each sampling in 7 replications at the A-set and in 14 replications at the B-set. The average values were used in the following discussion.

Apparent photosynthetic activity was measured in the wilted leaves under given field conditions by the modified Sachs method or the "leaf-punch method". Fifty leaf disks, whose total area was 57 sq. cm., were punched out from the matured laminae, excluding the large veins, of the leaves at the eighth-eleventh nodes from apex. These disks were oven-dried at 90° immediately after punching. The difference between the dry weight of leaf disks at the first punching and that at the second punching after two hours, gave approximately the net photosynthesis under the conditions where the plants were exposed, although some deviation should be induced mainly by decrease of assimilates in leaves by respiration and translocation. The mean value of the duplicate measurements was expressed by mg. dry weight/50 sq. cm./hr. A minute reduction of the leaf surface accompanied with wilting was neglected.

Results and Discussions

1. The growth: Effects of root excision on the growth in plant weight are set out in Fig. 1 and Tables 1 and 4. Growth difference, in dry weight as well as in plant height, between the A- and B-sets could be seen rather slightly for the first week of the treatment and became marked with development of the plants. At the end of the experiment the growth of plant at the B-set reached only 63 per cent in total dry weight and 79.5 per cent in plant height of the A-set (112 g. d.w., 127 cm.). Leaf growth was extremely depressed at the B-set throughout the experimental period because of reduction of fresh leaf or active leaf area with early senescence of leaves in the first week of the root excision. The treatment induced much depres-

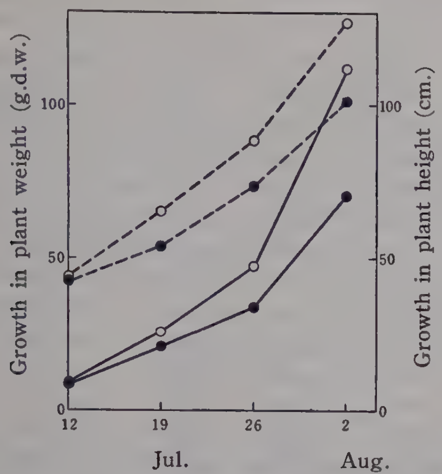


Fig. 1. Difference of growth in dry weight (continuous lines) and in plant height (broken lines) between the control plants (open circles) and the plants whose roots were partially excised (closed circles). The treatment was given on July 12.

sion of the fresh lateral root but none of the tap root. The subsequent recovery of the root system brought about only in a few days a new lateral root system which was of the same in dry weight as that of the control plant. The linear relationship with a regression line of $\bar{F}=207.8\ C_w-453.8$ was also demonstrated between total leaf area $\bar{F}$ (sq. cm.) and lateral root C_w (g.f.w.), as seen in tobacco plant[4]).

Net assimilation rates (NAR, mg.d.w./sq.cm./week)[3]), which indicates the efficiency of leaves in the growth rate, were as follows:

	July 12—19	July 19—26	July 26—Aug. 2
at the A-set	7.37	5.26	8.83
at the B-set	7.02	4.05	6.52

It is very clear that the NAR for the A-set always exceeded the NAR for the B-set. The significant depression of NAR in both sets from July 19 to 26 was rather caused by bad weather with insufficient solar radiation.

From the above facts, it could be considered that the effect of partial root excision results in the reduction of transpiring surface and that the rapid recovery of the root system from the injury may offset the unbalance of water economy of the plant within a short time. These two, conversely, give rise to a decrease of photosynthetic system and the large consumption of produced matter for the recovery of root system, and make worse the subsequent growth of the plant.

2. The variations in leaf water content: From the night of July 13, the second day after root cutting, to the evening of the next day, the time course of leaf water content was studied in leaves of about the same nodes as those used for net assimilation measurements (see section 3). The average value of duplicate measurements was expressed by leaf water index, mg. water/1 sq. cm. leaf area[4]). As was presented in Fig. 2, the effect of root cutting revealed itself in clear difference in the time course of leaf water index (cf. also Wilson *et al.*[7]). A maximum of leaf water index appeared just before sunrise (5 a.m.), i.e., 25 mg. H_2O at the A-set and 23.6 mg. at the B-set. The minimum, estimated from the curves in Fig. 2, could be seen at 2 p.m., with values of 22 mg. in the A-set and 18.5 mg. in the B-set. These values indicate the occurrence of extreme leaf water deficits at daytime.

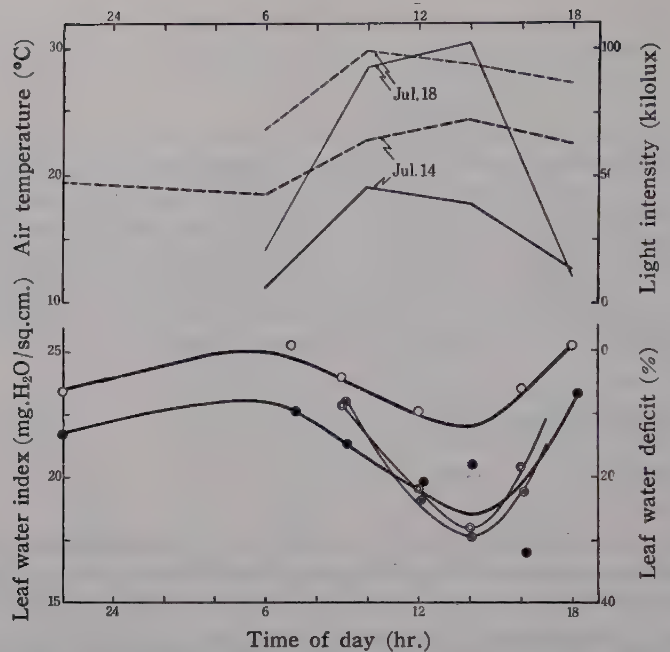


Fig. 2. Diurnal variations of a leaf water index or leaf water deficit on July 13–14 (thick lines: ○ the control, ● the treated) and on July 18 (thin lines: ◎ the control, ⊙ the treated). The above figures indicate light intensities (solid lines) and air temperatures (broken lines) were also shown.

As discussed in a previous paper[4]), leaf water amount of an intact plant can chiefly, if neglected small contribution of stem water, be determined by the active root/leaf area ratio ($C_w/\bar{F}$ ratio[4])) and by the transpiration rate, the latter being mainly affected by stomatal movement in case of a constant atmospheric saturation deficit. It was, however, difficult to find out any special relationship between transpiration rate and daylight intensities in the range of 10—75 kilolux (Fig. 5), though below 10 kilolux a relationship was observed between both measures (unpublished). —— concerning discussions in detail see section 3.

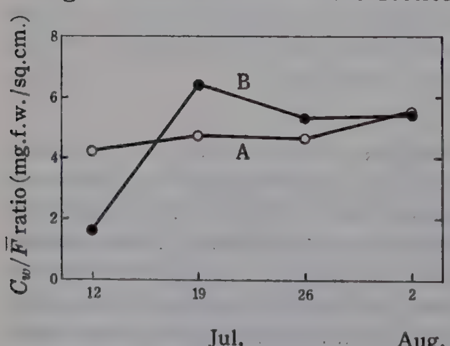


Fig. 3. Variations with time of the active root/leaf area ratio ($=C_w/\bar{F}$ ratio) in the A- and B-sets. Root excision was made in the B-set on July 12.

Fig. 3 shows the time trend of the $C_w/\bar{F}$ ratio (cf. also Table 1). With partial root excision on July 12, the ratio at the B-set was decreased to 1.6 in comparison with 4.2 at the A-set. This significant difference in the C_w/F ratio might bring

about the different absolute values of leaf water content on July 14 between the A– and B–sets. Six days after the root cutting (July 18) when the latter surpased rather the former in the $C_w/\bar{F}$ ratio, both the sets showed more or less the same diurnal rhythms in changes of leaf water index (see Fig. 2), under the conditions of intense light exposure of 100 kilolux and high air temperature of 30°.

No significant difference of soil moisture content could be observed between the A– and B-sets. On July 18, the soil moisture content at the A–set was 40.6 and 43.4 per cent on a dry weight basis at 10 and 20 cm. depths, respectively, and that at the B–set 38.3 and 40.4 per cent at respective depths. On July 24, the values were 58.9 and 62.7 per cent at the A–set, and 60.0 and 75.2 per cent at the B–set respectively. The maximum depth reached by the root system was 10—20 cm. on July 19 and 26. Therefore, it may be conclusive that between both sets appeared to be no difference in the resistance to water absorption from the rhizosphere.

3. Relation between net assimilation and leaf water deficit: In parallel with the determination of the time course of leaf water index, net assimilation of leaves was measured under a light-saturated condition over 25 kilolux, on July 14 and 18. The obtained results illustrated in Fig. 4 indicate clearly that the net assimilation decreased linearly with increase of leaf water deficit, irrespective of the kind of the sets. At little, less than 5 per cent, leaf water deficit, leaves assumed to have a maximum net assimilation of 7.2 mg.d.w./50 sq.cm./hr.

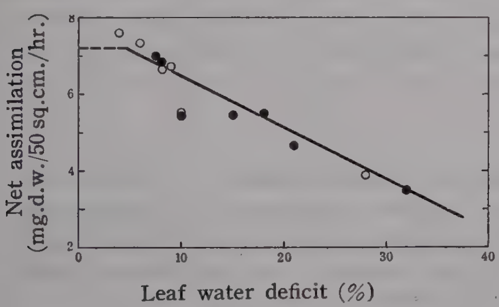


Fig. 4. Relationship between net assimilation and leaf water deficit. Open circles, the control; Closed ones, the treated.

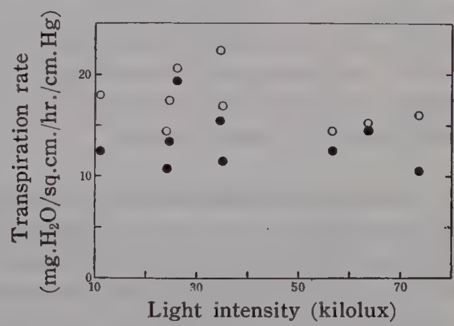


Fig. 5. Transpiration rates under strong light intensities. Measured in the field on July 14 and 18. Open circles, control plants: Closed ones, treated plants.

That stomata closure is induced with water deficiency of a leaf is generally believed, and this may limit the net assimilation. To clarify the stomatal behavior of wilted leaves, transpiration rate was investigated on July 14 and 18 in parallel with the net assimilation measurement by measuring loss in fresh weight of a leaf for 3 minutes just after detaching. The diurnal rhythm of transpiration rate was obscured by individuality of leaves and fluctuation with leaf age, even under the condition with clear rhythm of leaf water deficit. Fig. 5, where the transpiration rates were illustrated in a coordinate system against light intensity, shows a slight depression of transpiration rate at the B–set, without any clear relationship between these two measures (in the stronger light range above 10 kilolux). Generally speaking, there seemed to be no stomata closure with leaf water deficit in these sunflower plants, and the observed depression in net assimilation had not to be induced by the closing of stomata but directly by the water deficiency in mesophyll cells, as discussed by Dastur *et al.*[9]).

As for the influence of the partial root excision on the absorption of mineral nutrients, a total nitrogen content of the leaf was measured by a micro-kjeldahl method. Until July 26, one could hardly recognize any peculiar deviation in the values summarized in Table 2. This may suggest that the mineral nutrient absorption limited by the treatment did not reveal itself in the nitrogen deficiency in leaves

Table 2. Difference in total nitrogen content of leaves between the A- and B-sets (mg.N/g.d.w.).

Set \ Date	Jul. 12	Jul. 19	Jul. 26	Aug. 2
A	64.8	64.1	56.1	62.3
B	64.4	59.3	54.1	49.0

for the first two weeks and might not be directly responsible for the net assimilation depression discussed in the foregoing. A marked depression in leaf nitrogen content on Aug. 2, after three weeks from the root excision, remained to be studied, however.

In conclusion, under the conditions of intense illumination and high temperature, wilting occurs with a large amount of water consumption by plants to induce a depression of subsequent plant growth. The partial excision of root system gives rise to a severe temporary wilting in leaves at daytime, depressing the photosynthetic activity of leaves and consequently the total growth of the plant, until the root system recovers from the injury, or in other words, the active root/leaf area ratio again reaches a normal value.

4. **Theoretical elucidation of the depression in total growth:** For the quantitative elucidation of the above mentioned facts, matter production of the plants was calculated theoretically with combining photosynthetic acticity and the leaf water deficit, which could be obtained according to the following equation (see a previous paper[4]), page 18). Actual leaf water index (mg.H_2O/sq.cm.)

$$LWI = LWI_0 + (A_t \cdot C_w/\bar{F} - d \cdot T_l), \qquad \cdots\cdots(1)$$

where LWI_0 is the normal leaf water index: A_t, a coefficient of water absorption: $C_w/\bar{F}$, the active root/leaf area ratio (mg.f.w./sq.cm.): d, atmospheric saturation deficit (cm.Hg): T_l, transpiration rate (mg.H_2O/sq.cm./hr./cm.Hg) of leaf. And,

$$\text{relative leaf water deficit } (\%) = \frac{LWI_0 - LWI}{LWI_0} \times 100 \qquad \cdots\cdots(2)$$

Each factor concerned was determined as follows: (1) A_t was estimated with Equation (1), where being substituted a maximum (5 a.m.) and a minimum (2 p.m.) leaf water content on July 14 (Fig. 2), the amount of transpiration at a given time, and the active root/leaf area ratio on July 14 (Fig. 3). An average value of 2.4 of the coefficient was obtained. (2) Transpiration rates in Fig. 5 showed slight difference between the two sets. However, 16 mg.H_2O/sq.cm./hr./cm.Hg was adopted at both sets irrespectively to simplify the calculation. (3) The LWI_0 was determined as 25 mg.H_2O in average, despite its deviation from 22 to 27 mg. during the experiment. (4) The daily variation of saturation deficit was obtained from the 10 a.m. data at the Tokyo Central Meteorological Observatory, because they can represent the average

values at daytime (see Table 3). (5) Everyday's active root/leaf area ratio was assessed from Fig. 3.

Thus, the daily average values at daytime of the leaf water deficit varying with the active root/leaf area ratio were calculated as tabulated in Table 3. These values indicate that a severe water deficit might have occurred in the plants for the first 5 days of the experimental period at the B-set than at the A-set, while during July 19—28 with low solar radiation there might be hardly any water deficit at both sets except for the day with heavy saturation deficit, and that a considerable water deficit might have emerged at both sets in the daytime after July 29 when the weather turned fine, having a high saturation deficit.

Table 3. Daily variations of solar radiation, duration of illumination and atmospheric saturation deficit at the Tokyo Central Meteorological Observatory and of the average leaf water deficit and net assimilation under a light intensity stronger than 25 kilolux, calculated by Equations 1 and 2.

Date	Solar radiation cal./cm.^{2}/day	Duration of illumination (hrs.)		Saturation deficit at 10 a.m. cm.Hg.	Leaf water deficit % (*LWI* basis)		Net assimilation mg.d.w./50 sq.cm./hr.	
		>25 KL.	<25 KL.		Control	Treated	Control	Treated
Jul. 12	375	9	4.5	0.72	5.8	30.7	7.1	3.7
13	315	9	4.5	0.71	4.2	23.4	7.2	4.7
14	345	9	4.5	0.72	3 8	17.3	7.2	5.5
15	530	10.5	3	0.77	6.1	13.8	7.0	5.9
16	560	10.5	3	1.24	36.1	37.1	3.0	2.9
17	580	10.5	3	0.67	0	0	7.2	7.2
18	600	10.5	3	0.94	15.0	5.4	5.8	7.1
Jul. 19	360	9	4.5	0.96	16.3	0	5 6	7.2
20	150	7	6.5	0.36	0	0	7.2	7.2
21	155	7	6.5	0.26	0	0	7.2	7.2
22	335	9	4.5	0.50	0	0	7.2	7.2
23	200	7	6.5	0.28	0	0	7.2	7.2
24	425	9	4.5	0.61	0	0	7.2	7.2
25	225	8	5.5	0.34	0	0	7.2	7.2
Jul. 26	185	7	6.5	0.42	0	0	7.2	7.2
27	290	8	5.5	0.39	0	0	7.2	7.2
28	240	8	5.5	0.68	0	0	7.2	7.2
29	600	10.5	3	1.31	35.8	33.0	3.0	3.4
30	550	10.5	3	0.87	6.7	4.8	6 9	7.2
31	530	10.5	3	0.83	3.2	1.3	7.2	7.2
Aug. 1	625	10.5	3	0.93	8.6	7.7	6.7	6.8
2	460	10.5	3	1.23	25.9	26.9	4.3	4.2

Daily average values (at light intensities stronger than 25 kilolux) of net assimilation with varying leaf water deficits (Tab. 3, cols. 5 and 7) were estimated on the basis of the relationship illustrated in Fig. 4. The results are shown in Table 3, cols. 8 and 9. A photosynthetic acticity of 2.8 mg.$C_6H_{10}O_5$/50 sq.cm./hr., as a mean, was adopted, when light intensities were weaker than 25 kilolux and leaf water deficit may not occur as assumed from the data in Fig. 2. Leaf respiratory acticity of 0.8 mg.$C_6H_{10}O_5$/50 sq.cm./hr. was determined after Böhning *et al.*[8]). The duration of

illumination stronger than or weaker than 25 kilolux was calculated by using the data of the said Observatory (Table 3, cols. 3 and 4).

Respiratory activities of non photosynthetic organs at 25° were measured of 2.6 mg.d.w./g.d.w./hr. at the root system (water content 1130%) and of 2.3 mg. at the stem (water content 1069%) by a modified Boysen Jensen method. These activities may be rather high as compared with those after Kidd *et al.*[10]). Flower buds were estimated in respiratory activity to be the same as the stem. Although the water content of stem and roots may fluctuate in parallel with changes of that of leaves, unfortunately there are so far almost no observations as to how the variation in water content of them affects their respiratory activity. Correction for temperature effect on physiological activities were not made: hereto during the experimental period, the air temperatures at 10 a.m. fell in the range of 22°—31° and the mean soil temperatures at 10 cm. depth, 24°—29°.

The growth process, or dry matter reproduction, of the sunflower plants in both sets was traced by calculating the matter production with the values discussed above by the same method as reported before[3]). A fairly good coincidence between the observed growth and the calculated can be seen in Table 4 that illustrates the clear comparison between both sets. For example, on July 19, one week after starting the experiment, the observed dry weight and the calculated of the sunflower plants were 25.1 g. and 24.0 g. at the A-set, and 20.9 g. and 20.5 g. at the B-set, respectively.

Table 4. Comparison between the observed and the calculated dry weight (g./plant) in each organ. Figures in parentheses indicate the dry weight of dead leaves.

	Control plant							Treated plant						
	Jul. 12	Jul. 19		Jul. 26		Aug. 2		Jul. 12	Jul. 19		Jul. 26		Aug. 2	
	obs.	obs.	cal.	obs.	cal.	obs.	cal.	obs.	obs.	cal.	obs.	cal.	obs.	cal.
Leaf	4.6	11.3	11.0	16.9	18.2	31.6	33.8	4.6	9.1	9.2	13.2	16.8	26.0	34.9
	(0.1)	(0.1)	—	(0.3)	—	(5.6)	—	(0.1)	(0.3)	—	(0.3)	—	(2.7)	—
Stem	3.6	12.0	11.3	26.1	28.8	66.7	60.6	3.6	9.7	9.6	17.2	23.3	34.4	44.1
Flower	—	—	—	0.2	0.2	1.6	1.3	—	—	—	0.1	0.2	0.9	1.0
Root	0.5	1.7	1.7	3.8	5.0	6.4	6.4	0.36	1.8	1.7	3.1	4.2	6.6	8.3
Total	8.8	25.1	24.0	47.3	52.2	111.9	102.1	8.66	20.9	20.5	33.9	44.5	70.6	88.3

However, the excess of the calculated growth over the observed at both sets on July 26 might be induced by an overestimation of daytime's photosynthetic activity with no leaf water deficit. Moreover, some discrepancy between the observed and calculated at the B-set after July 26 may indicate the retardation of dry weight growth caused by the reduction of nitrogen content in leaves (see Tab. 2, Aug. 2).

At any rate, the mentioned agreement of the dry matter production seems to demonstrate the justice of the basic concepts and data applied to the theoretical analysis and synthesis of the growth process. The growth depression of the sunflower plants caused by removal of a part of root system resulted primarily from the depression of photosynthetic activity accompanied with increasing leaf water deficit for which the reduction of active root/leaf area ratio and severe environmental conditions are responsible, giving rise to an unbalance of water economy in the plants.

Summary

1. Under field conditions, applicability of equations of water economy obtained in a previous study[4]) was investigated in sunflower plants by causing a temporary wilting with partial excision of roots, 61 per cent of fresh lateral roots.

2. The treated plants could reach in dry weight only 63.1% of the control plants after three weeks from starting the experiment. No significant difference in total nitrogen content in leaves was detected between both plants at least for the first two weeks of the partial root excision.

3. A striking difference in time course of leaf water content was observed between the control and treated plants after two days of the root excision, but after 6 days the difference almost vanished (Fig. 2). The difference could be demonstrated by changes of the active root/leaf area ratio (Fig. 3).

4. Net assimilation of leaves decreased almost linearly with increase of leaf water deficit, while a closing of stomata could hardly be observed even under an intense light exposure.

5. The growth in dry weight of the control and treated plants was pursued by calculating the dry matter reproduction of the plants with Equations (1) and (2). The general agreement between the observed and calculated growth justifies the basic concepts and data adopted to the calculation. The growth depression by partial root excision can result chiefly from the depression of net assimilation caused by the temporary wilting of leaves.

The authors wish to express their thanks to Ass. Prof. S. Yokogi of the Tokyo University of Education for care of the material plants and his willing help.

References

1) Fukumoto, T., and Takahashi, M., Agric. and Hortic. **27**: 1219 (1952). 2) Humphries, E. C., Ann. Bot. **22**: 251 (1958). 3) Totsuka, T., and Monsi, M., Bot. Mag. Tokyo, **72** (855): 367 (1959). 4) ——, and ——, ibid, **73** (859): 14 (1960). 5) Weaver, J. E., Root Development of Field Crops. New York, p. 247 (1926). 6) Clements, F. E., Weaver, J. E., and Hanson, H. C., Plant Competition. Carn. Inst. Wash. Pub., 398 (1926). 7) Wilson, C. C., Boggess, W. S., and Kramer, P. J., Amer. J. Bot. **40**: 97 (1953). 8) Böhning, R. H., and Christel, A. B., ibid, **43**: 557 (1956). 9) Dastur, R. H., and Desai, B. L., Ann. Bot. **47**: 69 (1933). 10) Kidd, F., West, C., and Briggs, G. E., Proc. Roy. Soc., B. **92**: 368 (1921).

摘　　要

戸塚績*, 大島哲夫**, 門司正三*: ヒマワリの根の一部切除による生長の変化について.

水耕したタバコを実験材料として前報[3,4])で論じた水分経済の式を用いて, 野外条件下で栽培したヒマワリの生長を解析した. 播種後 39 日目に茎を中心にして半径 5 cm の円周で側根を切断し, 側根の61% を除去した. 処理後 3 週間目の処理区 (B) の乾物生長量は対照区 (A) の 63.1% に低下し, 伸長生長にも差がみられた (第 1 図). しかし, 葉の窒素含量は処理 2 週間後までは両区の間にいちじるしい差は認められなかった (第 2 表). 葉の含水量の日変化は切断 2 日後では (A) より (B) の方が常に低いが, 6 日後は恢復する (第 2 図). これは根の恢復による結果であって, 根と葉面積との割合 (active root/leaf area ratio) が葉の含水量変化に関係していることを示す (第 3 図). 含水量測定と平行して改良葉半法で測定した同化量は葉の水分欠乏が進行するにつれてほぼ直線的に減少した (第 4 図). 同時に測定した気孔の開度は強光下でもほとんど変化しなかった (第 5 図).

以上の諸事実にもとずいて, 生育期間中の気象条件を加味して葉の水分欠差を式 (1), (2) より算出し, さらにこの値と物質生産とを結びつけて生長量を算出したが, それは実測値とほぼ一致した. このことは根の一部切除による生長減退は, おもに葉の一時的しおれによる同化能率の低下した結果であることを裏書きする. (*東京大学理学部植物学教室 **富山県中新川郡水橋町 三成中学校)

Bot. Mag. Tokyo, **73**: 398—403 (October 25, 1960)

The Ratio of Chlorophyll *a* to *b* in the Plumule of *Nelumbo nucifera*

by Kiyonobu TOYODA*

Received April 6, 1960

In a previous paper[1]), the writer reported the existence of chlorophylls in the seeds of *Nelumbo nucifera* and some other angiospermous plants. On the chlorophyll formation, numerous works have been done, and the ratio of chlorophyll *a* to *b* in various leaves are known. On the ratio in the seed, however, almost no report has been submitted. The present paper is concerned with the ratio of chlorophyll *a* to *b* in the *Nelumbo* fruit.

Materials and Method

The materials investigated are as follows: the plumule in the various maturing stages[2]) of the fruits of *Nelumbo nucifera* Gaertner, the cotyledon of *Glycine Max* Merrill, normal leaf of *Nelumbo* and juvenile leaf which sprouted from the *Nelumbo* fruit in the laboratory.

Regarding the quantitative analysis of chlorophyll, various methods have been worked out. It is difficult in the case of *Nelumbo* plumule to separate chlorophyll *a* and *b* by column chromatography and to estimate them because of the small quantity. Various paper chromatographical methods[3, 4, 5]), are known, some of which are based on the theory that the area of the spot is proportional to the logarithm of the amount of the substance. In the chromatogram of the chlorophyll pigment, however, each spot on the filter paper does not necessarily represent a pure pigment; the density of the pigment in each spot is not similar, and the boundary of the spot is often unclear. Therefore, it is not easy to analyze chlorophyll pigment paper-chromatographically. Having some errors, paper chromatography is available for quantitative analysis of chlorophylls. The pigments of the samples were extracted with methanol-acetone mixture (1 : 1) under very weak and indirect light. At first the pigments of *Nelumbo* leaf were developed chromatographically with the solution of various amounts and the area of each spot was measured, followed by drawing a graph.

Besides the paper chromatography, the absorption curves were taken with Recording Spectrophotometer, and the quantitative estimation of chlorophylls was made with the aid of the simultaneous equations[6]), using absorbance (E) values at 663 and 645 mμ.

The transmittance[7]) of light into the *Nelumbo* fruit which is concerned with chlorophyll formation, was measured by an illuminometer (Tokyo Kôden), the method being as follows: Five holes of 5 mm. diameter were made on two paper boards. *Nelumbo* fruits were cut across the top, in which the plumule exists. The top pieces of the fruits were placed in the holes and fixed with gum. Then, the paper boards with and without the samples were illuminated with an incandescent lamp under the illumination of 1000 lux and transmittance was measured.

* Fujisawa Higher School attached to Nihon University, Fujisawa, Kanagawa Pref., Japan.

Results

In order to obtain the relationship between the amount of chlorophyll and the area of the spot, the pigment solution of *Nelumbo* leaf was applied on a filter paper in various dilutions and the size of the spots was measured. The result is shown in

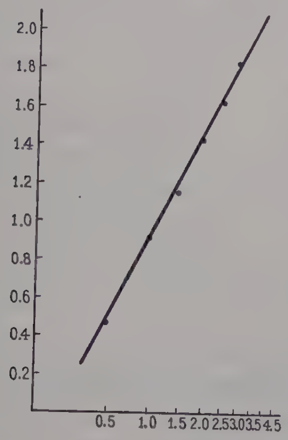


Fig. 1. The pigment of the *Nelumbo* leaf was developed in various amounts using methanol-acetone solution, and the areas of the spots were measured. The area of the spot in arbitrary units was plotted against the content of chlorophylls in logarithmic units.

Fig. 1, where the area of the spot was plotted against the content in logarithmic units. The line drawn in the figure shows that the area of the spot is proportional to the logarithm of the content of chlorophylls.

In the second maturing stage[2]) of *Nelumbo* fruit, the plumule becomes yellowish green. The pigment was extracted with a methanol-acetone mixture and was developed with carbon tetrachloride, toluene and xylene, showing yellowish green (YG) and bluish green (BG) spots or often only YG spot. The similar spots were observed with the samples up to the seventh maturing stage. The areas of BG and YG spots in the chromatograms of the *Nelumbo* plumule in various maturing stages were measured using a planimeter more than ten times. The ratios of BG spot to YG spot were calculated and the average values of the ratios were shown in Fig. 2. The ratios tended to become smaller with maturing.

The absorption curves of the pigments dissolved in acetone using the *Nelumbo* plumules of the fifth and seventh stages are shown in Fig. 3–A. The absorption curves of the pigment of YG spot which was cut off from the chromatogram of *Nelumbo* leaf and of the pigment of *Glycine Max*, in acetone solutions and with Cary spec-

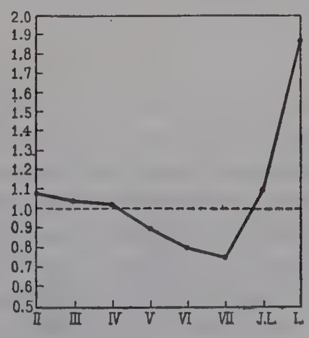
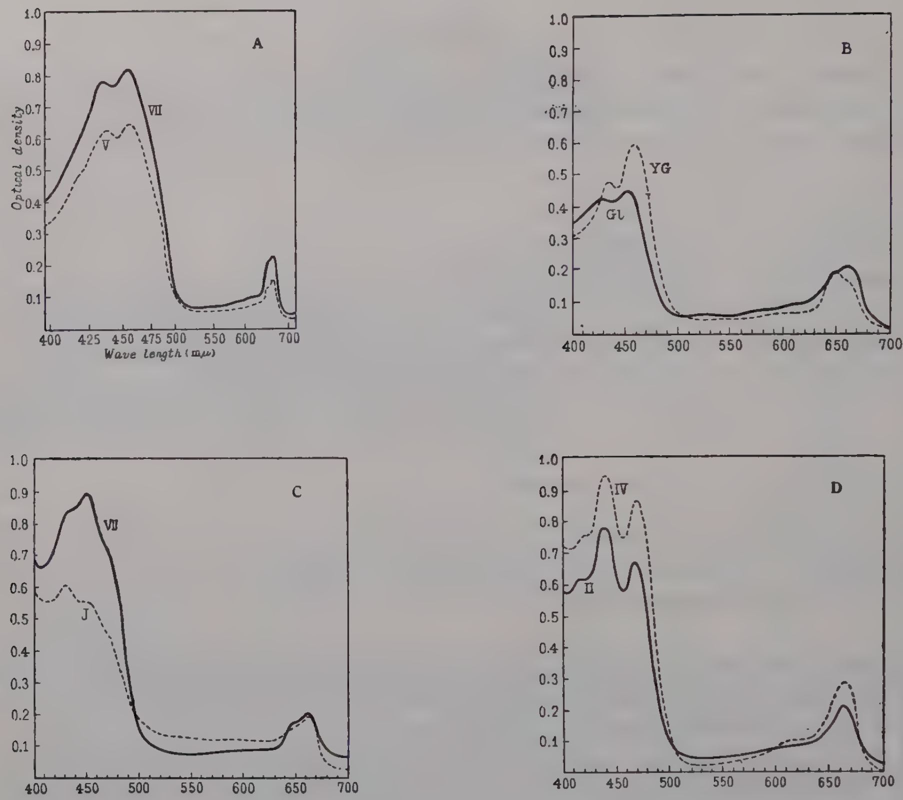


Fig. 2. The ratio of BG spot to YG spot in the chromatograms of *Nelumbo* plumule in various maturing stages, juvenile leaf (J.L.) and normal leaf (L).



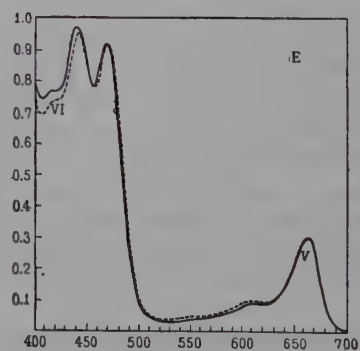


Fig. 3. Absorption curves. A) Using Beckman spectrophotometer (acetone solution), VII, *Nelumbo* plumule of the 7th stage V, the same of the 5th stage, B) Using Cary spectrophotometer (acetone solution), YG, YG spot in the chromatogram of *Nelumbo* leaf, Gl. *Glycine Max*, C) Do. VII, *Nelumbo* plumule of the 7th stage, J, juvenile leaf that sprouted from the fruit, D) Using Cary spectrophotometer (methanol solution), II, *Nelumbo* plumule of the 2nd stage IV, the same of the 4th stage, E) Do. V, the 5th stage VI, the 6th stage.

trophotometer, are shown in Fig. 3–B. The same of the *Nelumbo* plumule in the seventh maturing stage and juvenile leaf by acetone solution are shown in Fig. 3–C. The same of the *Nelumbo* plumule of the second and the fourth maturing stages and the fifth and sixth stages by methanol solution are respectively shown in Fig. 3–D, –E.

Calculating with the values of E 663 and E 645 using the simultaneous equations described above in the absorption curves of the *Nelumbo* plumules dissolved in acetone, the ratios of chlorophyll *a* to *b* were estimated in Tab. 1.

Table 1. The ratio of chlorophyll *a* to *b* calculated with the values of E 663 and E 645 using simultaneous equations.

Pigments	Ratio of chlorophyll *a* to *b*		
	Using Beckman spectrophotometer	Using Cary spectrophotometer	Average
Nelumbo plumule of 5th stage	0.86	—	0.86
〃 6th 〃	0.75	0.93	0.84
〃 7th 〃	0.72	0.85	0.79
Juvenile leaf of *Nelumbo*	—	1.08	1.08
Cotyledon of *Glycine Max*	0.58	0.86	0.72
YG spot of *Nelumbo* leaf	0.59	0.39	0.49

As the result calculated with the values of E 663 and E 645, it was clarified that YG spot in the chromatogram of *Nelumbo* leaf contains chlorophyll *a* and *b* in the ratio of about 1:2. The cotyledons of *Glycine Max* contain chlorophyll *b* more than chlorophyll *a*, and on the chromatogram of the pigment, which is scanty in chlorophyll *a*, only YG spot was detected.

In the absorption curve of chlorophyll pigment dissolved in methanol, though a shoulder at 645 mμ did not appear, distinct peaks were seen at 470 and 440 mμ (Fig. 3–D, –E). In the absorption curves of plumule was found that the ratio of E 470 to E 440 became gradually and steadily smaller with maturing and the results are shown in Fig. 4.

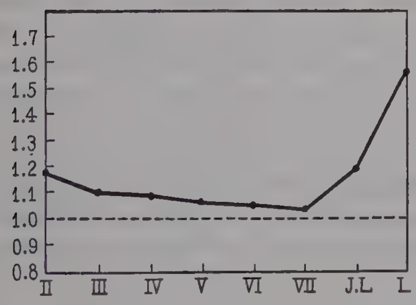


Fig. 4. The ratios of E 440 to E 470 in the absorption curves of *Nelumbo* plumule pigments dissolved in methanol using Cary spectrophotometer (see Fig. 3–D, –E). II—VII: Maturing stages of the plumule, J.L: juvenile leaf, L: foliage leaf.

The transmittance of light into the *Nelumbo* fruits which was measured by the method mentioned above is as follows:

Table 2. The transmittance of light into the *Nelumbo* fruits in the various maturing stages.

Maturing stages	1st	2nd	3rd	4th	5th	6th	7th
Transmittance (%)	0.5	0.3	0.2	0.1	0.1	0	0

Discussion

Generally it is considered that in the initiation of chlorophyll formation in normal leaf, protochlorophyll[8, 9, 10]) is first formed and subsequently chlorophyll *a* and *b* are formed. In the plumule of *Nelumbo* fruit the same phenomenon may also occur, but in the present experiment, the initial state of chlorophyll formation could not be observed. In the plumule of *Nelumbo*, protochlorophyll was hardly detected in the absorption curve with any of the acetone and methanol solution, and also in the chromatograms.

Comparing the ratios in the plumule of *Nelumbo*, it is clear that the ratio of chlorophyll *a* to *b* in the plumule became smaller progressively with maturing. That is to say, the amount of chlorophyll *a* seems at first larger than that of chlorophyll *b*, then chlorophyll *b* increases gradually and becomes larger than chlorophyll *a*. These facts suggest that in the plumule of the early stage, chlorophyll *a* is comparatively less formed than normal leaf on account of the sparseness of the available light (Tab. 1). Chlorophyll *a* in the plumule of the late stage was much less formed and became smaller than chlorophyll *b* owing to no illumination. In the juvenile leaf that came out from the *Nelumbo* fruit, the ratio of chlorophyll *a* to *b* became larger. It is, therefore, considered that more chlorophyll *a* was formed on account of indoor light. In the foliage leaf, the amount of chlorophyll *a* is much greater owing to intense and continuous illumination.

The absorption curve of the pigment dissolved in methanol, did not show any shoulder at 645 mμ, therefore it is difficult to calculate using the simultaneous equations. Acetone is an adequate solvent to see the shoulder of chlorophyll *b* at 645 mμ. In the methanol solution, however, distinct peaks were seen at 470 and 440 mμ. Although the bands of carotenoids overlap on these peaks, the peaks are considered to be due to chlorophyll *a* and *b*. Studying the ratios of E 440 to E 470 in the absorption curves of *Nelumbo* plumules in various maturing stages, it was found that these ratios became steadily smaller with maturing. In the juvenile leaf, the ratio became larger and in the foliage leaf, it became obviously much larger (Figs. 3-D, -E and 4). It is clear that those ratios have a close correlation with the ratio of chlorophyll *a* to *b*.

The writer wishes to express his sincere gratitude to Professor S. Hattori of the University of Tokyo for his kind guidance and invaluable advice. Further he thanks heartily to Professor S. Nagami of the Yokohama University for his kind guidance.

Summary

1. Paper chromatography is useful to some extent for quantitative analysis of chlorophylls.

2. The ratio of chlorophyll *a* to *b* in the *Nelumbo* plumule was calculated with the absorbance values at 663 and 645 mμ using simultaneous equations.

3. In the absorption curve of chlorophyll pigment dissolved in methanol, a shoulder of chlorophyll *b* at 645 mμ did not appear. Calculating the ratio of E 440 to E 470 in the absorption curve of *Nelumbo* plumule, however, it was found that these ratios became progressively smaller with maturing. It is considered that those ratios have a close correlation with the ratio of chlorophyll *a* to *b*.

4. In the *Nelumbo* plumule, the ratio of chlorophyll *a* to *b* was considerably small compared with that in normal leaf and it became gradually small with maturing, while in the juvenile leaf just after sprouting, it was larger than in the plumule.

5. The transmittance of light into the *Nelumbo* fruits of various maturing stages was measured. It was found that a very faint light is transmitted through the fruit-coat and seed-coat in the early maturing stage, but in the late stage the plumule is entirely cut off from the light.

References

1) Toyoda, K., Bot. Mag. Tokyo **72**: 159 (1959). 2) ——, J. Jap. Bot. **33**: 85 (1958). 3) Fischer, R. B., Parsons, D. S., and Morison, G. A., Nature **161**; 764 (1948). 4) ——, Parsons, D. S., and Holmes, R., ibid. **164**: 183 (1949). 5) Miyaki, K., Satake, K., and Hayashi, M., J. Pharm. Soc. Japan **7**: 249 (1951). 6) Mackiney, G., J. Biol. Chem. **140**:315 (1941). 7) Yaguwa, R., and Sudo, M., Oyo Kisho (Japanese) **1**: 114 (1946). 8) Gabrielsen, E. K., Physiol. Plantarum **1**: 5 (1948). 9) Shibata, K., J. Biochem. **44**: 147 (1957). 10) Frank, S. R., J. Gen. Physiol. **29**: 157 (1946).

摘　　要

豊田清修：ハスの幼芽におけるクロロフィル *a* と *b* との量比

1．ペーパークロマトグラフィーはクロロフィルの定量にある程度利用できる．

2．ハスの幼芽におけるクロロフィル *a* と *b* との量比を，663 mμ と 645 mμ とにおける光学濃度の値から，連立方程式によって計算した．

3．メタノール溶液によるクロロフィルの吸収スペクトルにおいては 645 mμ におけるクロロフィル *b* の肩は現われなかった．しかしハスの幼芽のスペクトルにおける E470 に対する E440 の比率を計算すると，これらの比率は成熟につれて漸進的に小さくなることを見出した．それらの比率はクロロフィル *b* に対する *a* の量比と密接な関連をもつものと考えられる．

4．ハスの幼芽においては，クロロフィル *b* に対する *a* の量比は，ふつうの葉にくらべてかなり小さい，そして成熟につれて徐々に小さくなる．しかし果実から発芽した幼葉ではその比率は幼芽より大きい．

5．いろいろの成熟段階のハスの果実で光の透過率を測定した．その結果，初めの成熟段階では，わずかの光が果皮と種皮とを透過するが，終りの階段では幼芽はまったく光から遮断されていることがわかった．(日本大学藤沢高等学校)

Bot. Mag. Tokyo **73**: 404–411 (October 25, 1960)

The Effects of Gibberellin on the Germination of the Seeds of *Sedum kamtschaticum* Fisch.

by Tadashi FUJII*, Sigeo ISIKAWA*, and Atsushi NAKAGAWA*

Received April 9, 1960

The growth-promoting role of applied gibberellin has been demonstrated on the responses, such as cell division, elongation, fruiting, flowering and seed germination. Various aspects of these problems were summarized in recent reviews by Brian[1]), and Stowe and Yamaki[2, 3]). Stimulation of seed germination by gibberellin was investigated recently. It has been known that the light requirement of certain seeds such as lettuce and tobacco was eliminated by the application of gibberellin[4, 5]). Lona[6]) pointed out, however, that gibberellin would not be expected to act on germination in the same way as red light. Bünsow and von Bredow[7]) and Poljakoff-Mayber *et al.*[8]) also suggested that the process of germination caused by gibberellin might not be the same as that caused by light.

In the meantime, the separation of the whole process of the germination into several physiological phases has been carried out by Isikawa[9]) and Isikawa and Fujii[10]). It was also shown by Toole *et al.*[11]) that three distinct stages were remarkable in the processes of seed germination, namely (a) imbibition of water, (b) cell elongation, and (c) increase in cell number.

The present paper is concerned with the effects of applied gibberellin on the germination processes of *Sedum* seed.

Material and Methods

The studies were performed on the light sensitive seed of *Sedum kamtschaticum* Fisch., a collected by Mr. Y. Yokohama in October 1959, at Mt. Nyugasa, Nagano Prefecture.

The gibberellin used was kindly given to the authors by Dr. Y. Murakami of the National Institute of Agricultural Sciences.

Gibberellin, in the amount necessary to produce the desired concentration in the medium, was added to the autoclaved agar medium just before its solidification. 0 7–0.8% agar-medium in which gibberellin was dissolved in various concentrations was poured into Petri dishes of 6.5 cm. in diameter. On the solidified surface, a filter paper wet with gibberellin solution in the corresponding concentration was placed directly, and 100 seeds were disseminated into each Petri dish. Next, each dish was wrapped in thick black paper, and placed in an incubator. The black cover was removed only when the irradiations were given on the seeds.

In the case of short exposures of germinating seed to gibberellin, the following treatments were carried out under the presence of blue light (fluorescent light filtered through four layers of dark blue cellophane). The seeds pre-soaked with water were removed from the agar-bed together with the filter paper under them and were placed still on the soaked filter paper between four sheets of dried filter paper to remove the humidity surrounding the seeds, and they were disseminated into Petri

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.

dishes containing the indicated concentration of gibberellin. And then Petri dishes were covered with black paper and returned to the incubator. After various durations of exposure to gibberellin, the gibberellin was washed off from the seeds with running water for 10 minutes. Thus, they were then allowed to germinate on the filter paper wet with water, placed on plain agar-bed.

The light treatments were similar to those described for *Nigella* seeds[9]. Red irradiation was obtained from the standard cool white fluorescent lamp through a filter of two layers of red cellophane, and far-red was separated from the incandescent lamp through a filter of two layers of red and two layers of dark-blue cellophane. A definite intensity of irradiation was obtained by regulation of the distance from the light source to the irradiated Petri dish and examined by a photometer. Temperature was controlled at 23° except when it was changed specially under the experimental necessity.

Results and Discussion

Preliminary experiments concerning the effects of gibberellin on germination indicated that the seeds of *Sedum kamtschaticum* Fisch. were induced to germinate by the application of gibberellin in continuous darkness and in constant temperature.

Dose-response experiments were set up first with gibberellin levels varying from 0.0025 to 200 ppm. As indicated in Fig. 1, the stimulative effect of gibberellin on the germination of *Sedum* seeds in their germination percentages was the strongest at 25 ppm., but it was not observed at 100 ppm. in gibberellin concentration. The germinated seeds treated with gibberellin in concentration of 25 ppm. were, however, eradicated as a result of the inhibited growth of their radicles after germination. Similar results were obtained to some other species[12–14].

It is interesting, however, that the short period applications of gibberellin in a fairly high concentration were remarkably effective in promoting germination. Namely, each lot of seeds presoaked for various times with 100 ppm. gibberellin in

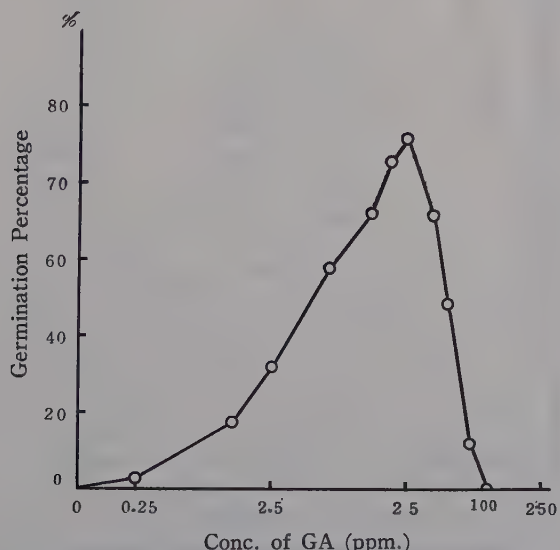


Fig. 1. Effect of gibberellin on germination of *Sedum* seeds in darkness.

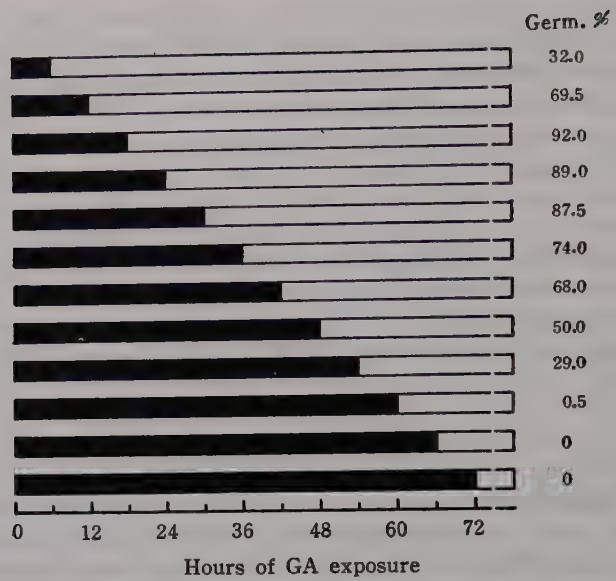


Fig. 2. Effect on *Sedum* germination of various lengths of gibberellin exposure. Gibberellin exposures were carried out from the beginning of imbibition with a concentration of 100 ppm.

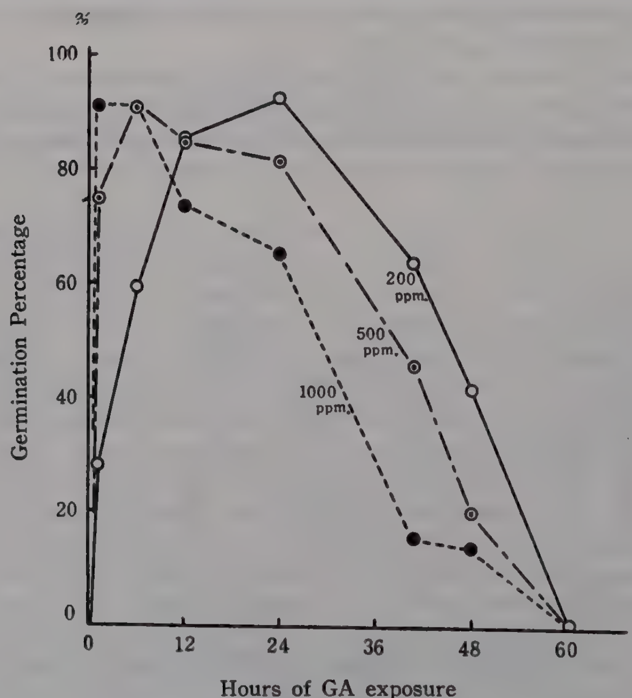


Fig. 3. Effect on germination of various lengths of gibberellin exposure at various concentrations. The treatment were carried out at 3 hrs. after the beginning of imbibition.

the dark, was transferred to a new plain agar bed respectively. In this experiment, the effectiveness of gibberellin treatment at 100 ppm. increased rapidly with the lenghthening of exposure during the first 18 hrs. after the start of imbibition. The germination percentage in an 18 hr.-exposure gave a maximum value (92%), and then it gradually decreased with the increase of exposure time. The complete loss of the promoting effect of 100 ppm. gibberellin was observed in case of continuous exposure longer than 60 hrs. from the start of imbibition (Fig. 2).

In the next experiments to observe the interrelations between the exposure time and the concentrations of gibberellin, seeds were exposed to gibberellin at various concentrations for various lengths of time at 3 hrs. after the beginning of imbibition. The effectiveness of gibberellin exposure increased at first, and then it gradually decreased as the exposure time was prolonged (Fig. 3). The maximum percentages of germination induced by the treatment of 200, 500 and 100 ppm. gibberellin were obtained with the exposures having the length of 24, 3 and 1 hr., respectively (Fig. 3). These experimental results suggested that the short application of gibberellin at high concentrations was effective to promote the preceding stage (germination-inductive stage) in the course of germination response, and inhibitive to the succeeding stage (maybe in elongation). In order to prove these suppositions on the actions of gibberellin for the germination of *Sedum* seeds, the following experiments were performed.

Several lots of *Sedum* seeds pre-soaked with water were respectively exposed for 24 or 6 hrs. to gibberellin of 100 ppm. in concentration, at various imbibition time. In the case of 24 hr.-exposure, a maximum value (more than 90%) of germination percentages was maintained during the first 36 hr. from the start of imbibition, and

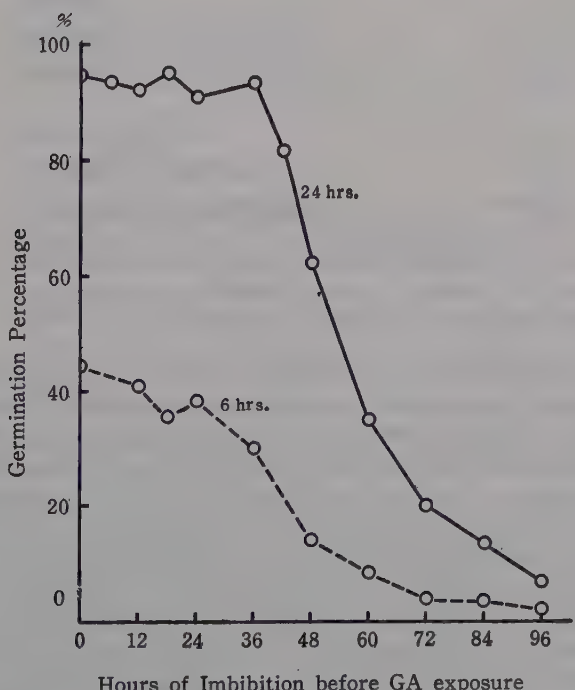


Fig. 4. Sensitivity of *Sedum* seeds to 24 and 6 hrs. exposure to 100 ppm. gibberellin.

then it gradually decreased with the lapse of imbibition time. Similar result was obtained for 6 hr.-treatment. However, the highest percentage of germination for this application was about 40% (Fig. 4). These sensitivity-curves to short application of 100 ppm. gibberellin were parallel to the effectiveness of red irradiation for the induction of germination. In *Sedum* seeds, a continuous irradiation of 1,500 lux for more than 24 hrs. was required to give a maximum percentage at 3 hrs. after the beginning of imbibition. But this maximum value was maintained during the first 24 hrs. from the start of imbibition, and then it gradually decreased with the lapse of imbibition time (Fig. 5). These results indicated that gibberellin of fairly high concentrations could substitute red light to the induction of germination of *Sedum* seeds.

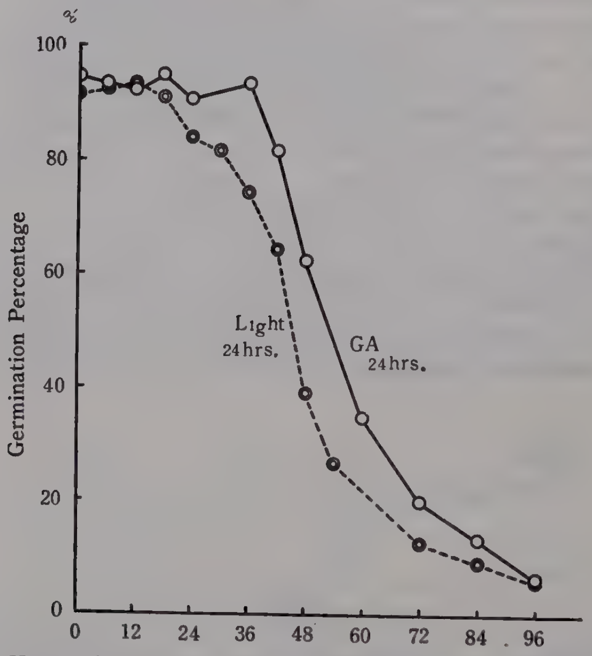


Fig. 5. Sensitivity curves of *Sedum* seeds to light- or gibberellin-exposure of 24 hrs. respectively.

While, it was shown with light sensitive seeds that a succession of several alternate red and far-red irradiations resulted in a promotion of germination when the alternation ended with a red irradiation and resulted in an inhibition when the irradiation treatment terminated with an exposure to far-red irradiation. The light action on the germination of *Sedum* seeds was also repeatedly reversible (Table 1). After 3 hrs. passed since imbibition had begun, ten lots of *Sedum* seeds were irradiated for 1 hr. with red of 1,500 lux and one lot was returned to darkness. The remaining 9 lots were immediately irradiated for 1 hr. with far-red of 1,500 lux on the surface of Petri dishes and again one lot was returned to the dark. The 8 remaining lots then received another 1 hr. red irradiation and one lot was returned to the dark, and so on until all lots had been returned to darkness (Table 1). The chemical promotion of gibberellin, however, was not reversed by far-red irradiation (Fig. 6). These results suggested that gibberellin did not specially act on the light receptor,

Table 1. Effect of a succession of several alternate red and far-red irradiations at 3 hrs. after the beginning of imbibition.

Treatment	Germ. %
R*	33.5
R + FR**	1.0
R + FR + R	43.5
R + FR + R + FR	6.0
R + FR + R + FR + R	56.0
R + FR + R + FR + R + FR	4.5
R + FR + R + FR + R + FR + R	61.5
R + FR + R + FR + R + FR + R + FR	8.5
R + FR + R + FR + R + FR + R + FR + R	73.0
R + FR + R + FR + R + FR + R + FR + R + FR	11.5

* Red light for 1 hr.
** Far-red for 1 hr.

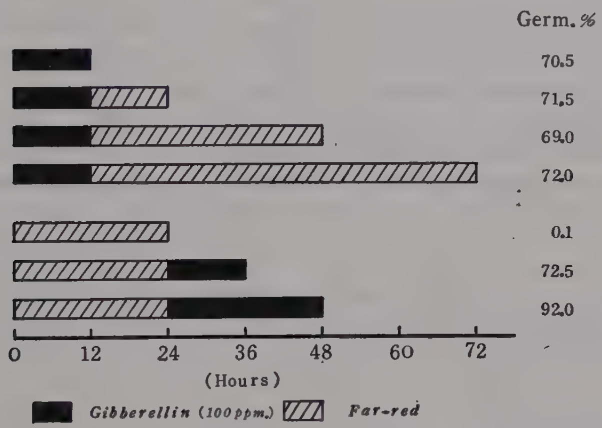


Fig. 6. Effect on far-red inhibition of gibberellin exposure.

but rather as a general germination stimulator, as suggested by Hayashi[15]), Bünsow and von Bredow[7]) and Poljakoff-Mayber *et al.*[8]).

On the other hand, to give a convincing proof of the inhibitive effects of 100 ppm. gibberellin application to the succeeding stage in the course of germination response, the combined treatments of the red irradiation (1,500 lux, for 18 hrs.) and a short application (24 hrs.) of gibberellin were performed at 23°. Namely, each lot of seeds irradiated by the red light for 18 hrs. from 3 hrs. after the beginning of imbibition, was exposed to 100 ppm. gibberellin, by varying the lengths of the interval between the light and gibberellin-treatment. The results were shown in Table 2. The red irradiation for 18 hrs. gave 76.5% germination, unless short applications of gibberellin

Table 2. Inhibitory effect of gibberellin in the succeeding stage in the course of germination.

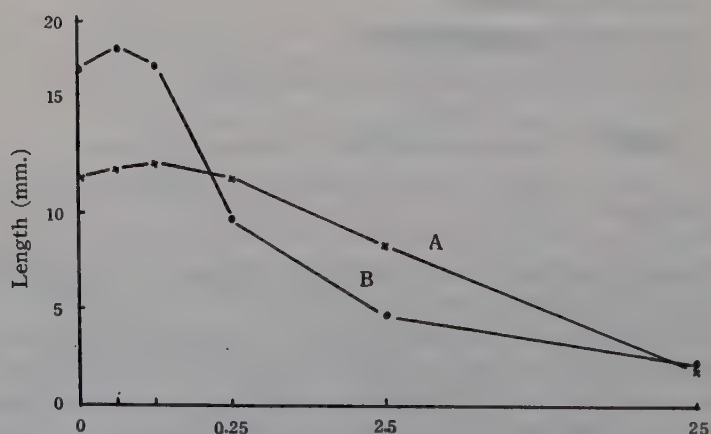
Gibberellin exposure (hours)	Hours between red irradiation and gibberellin-treatment								
	0	3	6	12	18	24	30	36	48
24	90.0	81.0	90.5	86.0	82.0	74.0	55.0	56.0	32.5
36	64.0	65.0	62.0	62.0	52.0	48.0	—	33.0	6.0

Red irradiation of 18 hrs.: 76.5% germination.

were made. The inhibitive effect, however, occurred when gibberellin treatments were carried out after the red irradiation, and increased as the interval was prolonged.

From these experimental results, it was suggested that high concentrations of gibberellin were unfavorable to the development following the germination-inductive stage. It is an important feature that the separation of the whole process of the germination into these two physiological stages by the actions of gibberellin could provide a new technique for the researches on germination.

Next, to observe the effects of gibberellin on the development following the germination-inductive stage, the intact radicles (about 1 mm.) induced to germinate under the light were used as a substitution, and displaced into Petri dishes containing the indicated concentrations of gibberellin. Some of them were placed for 6 days under the light and the others, in darkness and then they were measured their lengths. These results indicated that 0.25 ppm. concentration under the light, and 0.0025 ppm. in darkness were favorable for their elongation (Fig. 7). In a number of species whose seed germination was not inhibited by gibberellin even of a fairly high concentration, it was conceivable that the development following the germination-inductive stage of these species were not sensitive to gibberellin inhibition.



A. Lengths of radicles in darkness
B. Lengths of radicles under the light.

Fig. 7. Effects on the radicle of gibberellin at various concentrations.

Summary

1. Seeds of *Sedum kamtschaticum* Fisch. are induced to germinate with the application of gibberellin even if they have not been irradiated by light.

2. A continuous contact to gibberellin at a concentration of 100 ppm. is completely inhibitory to the germination of *Sedum* seeds. It is noteworthy, however, that a short application of a fairly high concentration of gibberellin is remarkably effective to promote the preceding stage (germination-inductive stage) in the course of germination on response, and is inhibitive to the development following the germination-inductive stage. In the *Sedum* seeds, high concentration of gibberellin is favorable for the induction of germination, and low concentration is favorable for their development.

3. Gibberellin-sensitivity to short application of high concentration is parallel to red light sensitivity curve. These results indicate that gibberellin can be substituted for red light in promoting *Sedum* germination. The fact that this chemical promotion with gibberellin is not reversed by far-red irradiation, however, suggests that the process caused by gibberellin may probably not be the same as that caused by red light.

References

1) ——, Biol. Rec. Cambridge Phil. Soc., **34**: 37 (1959). 2) Stowe, B. B., and Yamaki, T., Ann. Rev. Plant Physiol., **8**: 181 (1957). 3) Stowe, B. B., and Yamaki, T., Science **129**: 807 (1959). 4) Kahn, A., Goss, J. A., and Smith, D. E., Science **125**: 645 (1957). 5) Ogawara, K., and Ono, K., Proc. 1st Japanese Gibberellin Symposium 9–10 (in Japanese). (1957). 6) Lona, F., Ateneo Parmense **27**: 641 (Ber. wiss. Biol., **117**: 320 (1958)). 7) Bünsow, R., and Bredow, K. von, Biol. Zbl., **77**: 132 (1958). 8) Poljakoff-Mayber, A., Evenari, M., and Neumann, G., Bull. Res. Counc. Israel., D, **7**: 99 (Ber. wiss. Boil., **132**: 63 (1959)). (1958). 9) Isikawa, S., Bot. Mag. Tokyo **70**: 264 (1957). 10) Isikawa, S., and Fujii, T., Abstracts of Papers Presented at the 24th Annual Meeting of the Botanical Society of Japan, (in Japanese) (1959). 11) Toole, E. H., Hendricks, S. B., Borthwick, H. A., and Toole, V. K., Ann. Review of Plant Physiol. **7**: 299 (1956). 12) Brian, P. W., J. Roy. Soc. Arts **106**, 425 (Ateneo parmense 29.), (1958). 13) Gray, R. A., Plant Physiol. suppl. **33**: xl (1958). 14) Izard, C., and Hitier, H., Compt. rend. **246**: 2659 (1958). 15) Hayashi, T., J , Agr. Chem. Soc. Japan **16**: 386 (1940).

摘　　要

藤伊正・石川茂雄・中川篤:　キリンソウ種子の発芽に対するジベレリンの影響について

キリンソウ種子は光発芽種子であって，じゅうぶんな発芽を与えるためには，24 時間以上の連続照射を必要とする．しかし従来タバコ，レタスなどの種子で報告されているように，この種子もジベレリンを与えることにより，暗黒でも発芽が誘起される．しかし発芽過程の進行にともない，ジベレリンに対する反応も変化することが考えられ，発芽の全過程にわたって種子をジベレリン溶液中に置いた場合，ジベレリンが発芽のある段階を促進すると同時に他の段階を抑制し，その効果において，両者が相殺するという懸念が生ずる．それ故ジベレリンの真の発芽誘起を知るべく，ジベレリンの短時間処理を試みた，連続的処理においては最適濃度が 25 ppm であり，100 ppm ではまったく発芽を誘起しないにもかかわらず，短時間処理においては，1000 ppm の高濃度においてさえもいちじるしく発芽を誘起することを見出し，更に発芽の後段階ではジベレリンがかなりの低濃度においても阻害的に作用することを明らかにした．（東京教育大学理学部植物学教室）

Bot. Mag. Tokyo **73**: 412—416 (October 25, 1960)

Studies on the Mechanism of Seismonastic Leaf Movement in *Mimosa pudica* L.

I. Existence of Irritability in the Upper Half of the Main Pulvinus*

by Reizo AIMI**

Received April 22, 1960

Regarding the mechanism of the seismonastic movement of *Mimosa* leaf, the hypothesis which was presented by Pfeffer[3]) has been widely accepted as an estiblished theory so far. Namely, the bending movement of *Mimosa* leaf takes place by the diminution in turgidity of the tissue of the lower half of pulvinus upon stimulation. The loss of the turgidity results in an equilibrium change of tissue tension between the upper and lower halves of the pulvinus and causes a bending down of the leaf. In this process, only the lower half of the pulvinus is concerned.

According to the writer's observation, however, any significant differences in the protoplast of the pulvinus cells were hardly recognized. Similar results were also reported by Yamamoto[4]) and others. Hence doubt was cast as to the validity of the hypothesis which claimed the presence of irritability merely in the lower half of pulvinus. If this hypothesis be probable, it seems rather difficult to reconcile morphological similarity in the protoplast of the upper and lower halves of pulvinus with the localized existence of irritability in the lower half of the pulvinus. Furthermore, no rigorous experimental proof has been given thus far to the absence of irritability in the upper half of pulvinus. Hereupon it appeared to be of importance to re-examine the existence of irritability of the upper and lower halves of pulvinus separately. Precise experiments were, then, carried out with a leaf having either the upper or lower half of the pulvinus.

Method and Materials

According to the writer's tentative experiments by means of a routine kymograph, slight response appeared to exist even in a leaf having only the upper half of the pulvinus upon stimulation. This method, however, was not suitable to trace the very reduced movement of the leaf, because of (1) presence of a considerable frictional resistance between the smoked paper and the pen of writing lever, (2) insufficiency in magnifying power of the movement. Hence a more sensitive recording apparatus was constructed using an optical lever system and this was employed in this experiment.

Fig. 1 shows a diagram of the apparatus used. A common kymographion was placed in a dark box. A lever, which was equipped with a small mirror at one end, was set up level to the stand. At the other end of the lever, a fine cotton thread which was untwisted and slightly coated with vaseline, was tied. The other end of

* The content of this report was presented at the monthly meeting of the Botanical Society of Japan on October 1944, and its summary is described in the Botanical Magazine[1]) and also in Kagaku[2]).

** National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo, Japan.

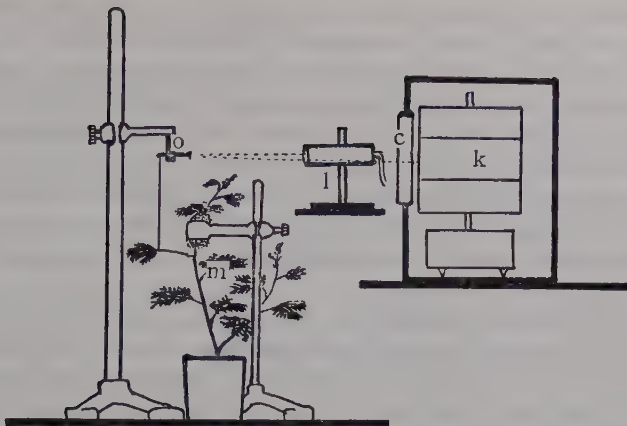


Fig. 1. Diagram showing the whole view of the apparatus used. m, *Mimosa* plant; o, Optical lever; c, Cylinder lens; k, Kymographion; l, Light source.

the thread was connected to the apical end of the petiole which was kept so as to be in parallel with the lever. A light beam from the light source reflected at the mirror, passed through the cylinder-lens of the dark box, and focused on photographic paper which was wound round the drum of the kymographion. The vertical movement of the leaf was conveyed to the lever system. As shown from this mechanism, in order to record the vertical movement of the leaf satisfactorily, the leaf petiole must be set up so as to be almost parallel to the lever. Hereupon the downward movement of the leaf will be recorded as an upward curve on the photographic paper, or *vice versa*. The magnitude of this lever system was approximately 4.6.

Experimental procedure Primary pulvini of fully developed leaves of a well-grown plant of *Mimosa pudica* L. were used as material. Several hours before experimentation, plants were transferred from the culture field into a room in order to make them accustomed to indoor conditions.

The operation removing the half part of the primary pulvinus was carried out using a sharp razor blade, retaining the vascular bundle which pierces through the middle part of the pulvinus. The cut surface of the pulvinus was coated with liquid paraffin so as to prevent the operated pulvinus from drying due to loss of the tissue fluid.

After this treatment, the *Mimosa* plant was carefully set up to the apparatus and allowed to rest for about one hour prior to the experiment to eliminate the stimulation effects received during the treatment.

Stimulation was made by depositing a drop of ethyl ether upon the pulvinus.

Further details regarding method and technique will be described in each pertinent section.

Results and Discussion

(1) *Existence of irritability in the lower half of pulvinus.*

It has been well known that even if the upper half of the pulvinus is cut away, the remaining half (i.e. the lower half) will still function satisfactorily, reacting to

stimuli in a weakened but otherwise normal fashion. Accordingly, experiments were first carried out with such a leaf in order to determine whether or not the new device is suitable enough to record the reduced leaf movement, in addition to determining the behavior of the irritable movement in the operated leaf.

Cutting away the upper half of the pulvinus for preparing the leaf having only the lower half of pulvinus, the leaf was bent down by the shock of the treatment. After a while, however, the leaf came up slowly, and almost attained to the initial position, and leaflets began to re-open. Such leaves which recovered to this state were capable of reacting normally again to stimuli. All the experiments were carried out with these leaves.

A typical curve of the movement of a leaf having only the lower half of pulvinus is shown in Fig. 2. The sudden bending movement occurred upon stimulation. The leaf then returned to the initial position within a rather shorter period than that displayed by the intact leaf, which usually requires about 15 minutes for recovery.

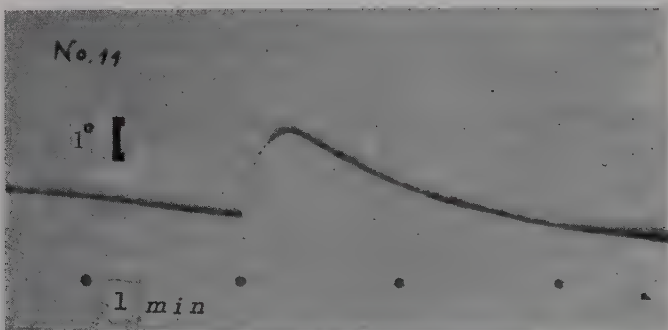


Fig. 2. Curve showing the movement of a leaf having only the lower half of the pulvinus.

From the fact that the downward movement occurs upon stimulation, it may be clear that the contraction of the pulvinus tissue takes place.

Observing the curve, we may realize that the base-line itself was descending continuously. This indicates that the leaf was erecting itself continuously, due to the tissue tension in the pulvinus of the remaining lower half. Despite this, the irritable bending movement occurred quite independently upon stimulation. In other words, there exist two kinds of bending forces within the same half of pulvinus. One is due to the contraction of the pulvinus tissue, the other is due to its extension. The former is revealed only when the leaf is stimulated, but the latter always exist apart from irritability, so far as the pulvinus maintains its own tissue tension. The author would like to term the former " irritable bending force ", and the latter, " non-irritable bending force ".

From the experiments in this section, the phenomenon of the irritable bending movement of the leaf having only the lower half of the pulvinus was clarified. At the same time, it was ascertained that newly designed apparatus is suitable enough for the recording of very reduced movements made by the operated leaves.

(2) *Existence of irritability in the upper half of pulvinus.*

From the foregoing experiment, it was ascertained that the apparatus used was capable of recording even the most reduced movements of the operated leaf. The experiment in question, examining the existency of irritability of the upper half of

the pulvinus, was then carried out with a leaf having only the upper half of the pulvinus.

In advance of the experiment, the cutting operation of the lower half of the pulvinus was made in the same manner as in the case of operating on the upper half of the pulvinus. In this case, however, when the lower half of the pulvinus was cut off, the leaf bent downward intensely owing to the tissue tension of the remaining upper half of pulvinus and it could come back again no more. Then, the leaf was inverted by bending the stem which attached this leaf so far as its petiole became to level, as there was no other suitable method to bring the petiole to level. In this position, the plant was fixed to the stand with crumps. Accordingly, this time, the remaining upper half of pulvinus came to the lower side. So the position of the upper half of pulvinus became to the same as the case of the lower of pulvinus in the normal position of the leaf. Consequently the relationship between the movement direction of the leaf and the contraction of the pulvinus also became to the same as the case of the leaf having only the lower half of pulvinus.

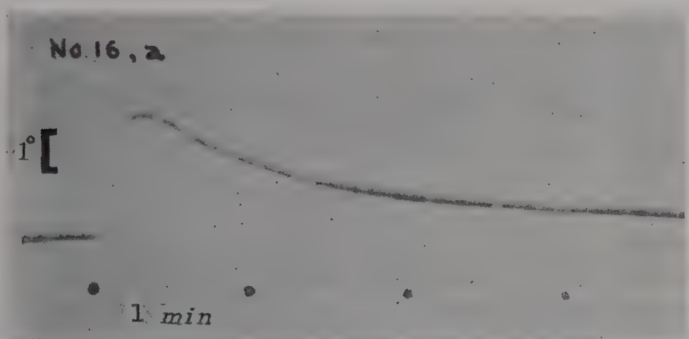


Fig. 3. Curve showing the movement of a leaf having only the upper half of the pulvinus.

Fig. 3 shows a typical curve of the irritable movement of an inverted leaf having only the upper half of the pulvinus. This curve is similar to that obtained by the leaf which is not inverted and have only the lower half of pulvinus, as shown in Fig. 2. The upward course of the curves in the figures indicates the downward movement of the leaf in the experimental positions and consequently it points out the contraction in the remaining half which is an upper side of the pulvinus thus situated. The mode of movement, therefore, of the leaf having only the upper half of the pulvinus was just like that of the leaf having only the lower half of the pulvinus.

From these experiments, there is no doubt that irritability exists even in the upper half of the pulvinus, as well as in the lower half. Consequently the hypothesis that only the lower half of the pulvinus is sensitive should be discarded.

Notwithstanding that current opinion favors the hypothesis that only the lower half of the pulvinus is sensitive, there were several observation suggesting the presence of irritability of the upper half of pulvinus. For instance, Bert[5]) observed that the *Mimosa* leaf from which the lower half of pulvinus was cut away moved about 5° upon stimulation. Lutz[6]) concluded that the diminution in volume of pulvinus cell occurred not only in the lower half but also in the upper half of pulvinus. Unfortunately these noteworthy findings have been left without any particular attention being paied by others up to now.

Summary

Regarding the mechanism of the seismonastic movement in *Mimosa* leaf, the hypothesis that only the lower half of the pulvinus is sensitive, has been widely accepted as an established theory thus far. Then, the existence of irritability of the upper half of the pulvinus was re-examined precisely using newly devised apparatus with optical lever system. As the results of experiments, it was clarified that the upper half of the pulvinus has irritability (contractibility) as well as the lower half.

A series of this research was conducted at the Botanical Institute of Tokyo University of Literature and Science (present Tokyo University of Education) under the cordial guidance of Professors Masuta Matsubara and Gihei Yamaha. The writer wishes to express his deepest gratitude to them and also to Professor Tomoo Miwa who gave kind criticism to this study.

References

1) Aimi, R., Bot. Mag. Tokyo **69**: 95 (1944). 2) ——, Kagaku **14**: 321 (1944). 3) Pfeffer, W., Pflanzenphysiologie (1904). 4) Yamamoto, S., Jap. J. of Med. Sci. Biophys. **5**: 148 (1938). 5) Bert, P., Mem. Soc. Sci. phy. et nat. de Bordeaux **4**: 11 (1866). 6) Lutz, C., Ztsch. f. Bot. **3**: 289 (1911).

摘　　要

相見霊三：　オジギソウの葉における傾震性屈曲運動の機構に関する研究.
(I) 主葉枕上半部における興奮性の有無について.

オジギソウの葉が刺戟を受けて屈曲運動を起す際，従来広く行なわれている考え方は，葉枕（主葉枕）の下半部が興奮収縮し，上半部組織の張力と相まって，下方に向う屈曲運動が起るもので，この際，刺戟に対し興奮性（被刺戟性）を有するのは下半部のみであるとされているようである．しかし，葉枕の上半部と下半部を構成する柔細胞の原形質をくらべてみると，下半部は興奮性を有するのに，上半部にはないと考えられるほど本質的な差違は見出し難い．そこで葉枕上半部の興奮性の有無を，光のてこを用いた運動描写装置によって追試してみた．その結果，上半部も下半部と同様，刺戟によって収縮する興奮運動を起し，興奮性が存在することを確めることができた．（農林省農業技術研究所）

Bot. Mag. Tokyo, **73**: 417—421 (October 25, 1960)

The Effect of Cobalt on the Growth of Pollen II Differential Acquisition of Cobalt-60 in the Style of *Lilium longiflorum*

by Yoshio YAMADA*

Received April 27, 1960

It has been shown in a previous paper[1]) that cobalt, given as sulphate, increases the rate of oxygen uptake of pollen grains and promotes the germination and growth of pollen of *Lilium longiflorum*. It has also been shown that cobalt can prevent the inhibitory effect of ethylenediamine tetraacetate and 8-hydroxyquinoline on the germination and growth of pollen, and suggested that this metallic ion may be, at least in *L. longiflorum*, an essential trace element.

In continuation of this work, the present experiments were designed to examine, by the use of radio-active cobalt (Co^{60}), the distribution of cobalt in various floral tissues of *L. longiflorum* at various stages in flower development and to know whether the pistil differs from the other floral tissues in the acquisition or utilization of cobalt.

Material and Method

Material: A differential acquisition of Co^{60} in various parts of flower was studied with *Lilium longiflorum* at various stages of flower development. When the flower buds grow up to the size desired, the stems after being severed from the roots, were placed in a vessel with 200 m*l*. of well-water containing carrier-free Co^{60} as $CoCl_2$. In each culture inorganic Co^{60} was given at an activity $3.83\times10^{5}\sim4.10\times10^{5}$ counts/m*l*./min., under the standard counting conditions. The amounts of Co^{60} given to the experimental plants in this work did not affect their growth rate. After 4 days' cultivation, samples of plants were taken for radio-assays for cobalt and the amounts of radio-isotopes remaining in the solution were determined. In the present study, flower buds of equal length were used as far as possible in order to eliminate individual differences in the physiological state. All experiments have been carried out in July and August, 1959.

Preparation of Samples: In each experiment the flower buds of seven plants were pooled. The tissues used for measurement were stigma, style, ovary, pollen grains (or anther), filament and perianth. Stem and leaf were also used as control material. Immediately after weighing, small pieces of each tissue, except the pollen grains, weighing about 1 g. were homogenized in deionized water to make a 4 m*l*. suspension. Each suspension was taken into a small polyethylene tube in order to determine its radio-activity.

Assay of Radio-activity: Radio-activity was measured using a well-type scintillation counter. The same scintillation counter was used throughout the present experiments and no significant changes of sensitivity were noted during the experimental period. All counts were corrected for background and radio-activity decay. All values were expressed in counts per minute per gram tissue (fresh weight).

* Biological Institute, Faculty of Liberal Art and Education, Gunma University, Maebashi Japan.

Results

Each experiment was repeated twice. Similar trends were observed in the duplicate experiments, but the measured values of the actual acquisition of Co^{60} in certain tissues, i. e. leaf and perianth, showed slight variations. These variations are probably due partly to the difference in the rate of absorption and partly to the difference in total weight of plants. The general physiological state of the plants might also affected the results obtained.

Experiment 1. Studies with the young flower buds.

After administration of Co^{60} for 4 days, the various parts of the flower, from a bud 7 cm. in length, were taken for radio-assays for cobalt. The distribution of the isotope is shown in Fig. 1 and the data reveal that cobalt is widely distributed throughout the plant tissues, except for the anther. In young flower buds, the stigma

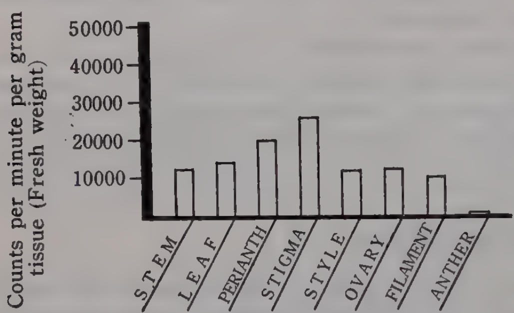


Fig. 1. Concentration of administered Co^{60} in various tissues of the young flower bud.

and perianth contained higher concentrations of cobalt, whereas the style, ovary and filament contained this element at lower and almost constant concentration. It is interesting that the anther including pollen grains does not concentrate Co^{60} to any appreciable extent in young flower buds. The concentration of cobalt in the stigma was about 2 times greater than that in the leaf and stem. Apparently there was a marked acquisition of this element in the stigma of young flower buds.

Experiment 2. Studies with the mature flower buds.

After administration of Co^{60}, the floral tissues, from a bud 15 cm. in length and about 3 days before anthesis, were taken for radio-assays for cobalt. The results are reported in Fig. 2. In the mature flower buds, there was a marked acquisition of Co^{60} in the two major conducting tissues, stigma and style, and to a lesser extent, in the ovary, anther, filament and perianth. The latters showed the same degree of acquisition. However, a significant increase in the Co^{60} acquisition in the anther including pollen grains was noted in mature flower buds. The concentration of Co^{60} in the stigma and style was 10 to 20 times greater than that in the leaf and stem. The very high concentration of Co^{60} in the style was only comparable with that in the stigma. Comparing these figures with the data mentioned in Exp. 1, it will be found that cobalt concentrates in the style to a greater extent in mature flower buds than in young ones.

Experiment 3. Studies with the open flowers.

After administration of Co^{60}, the open flowers of which anthers had already dehisced were taken for radio-assays for cobalt. Results are shown in Fig. 3. The most marked acquisition was observed in the style, stigma, perianth and ovary; the least activity was found in the filament and pollen grains. Only very small amounts

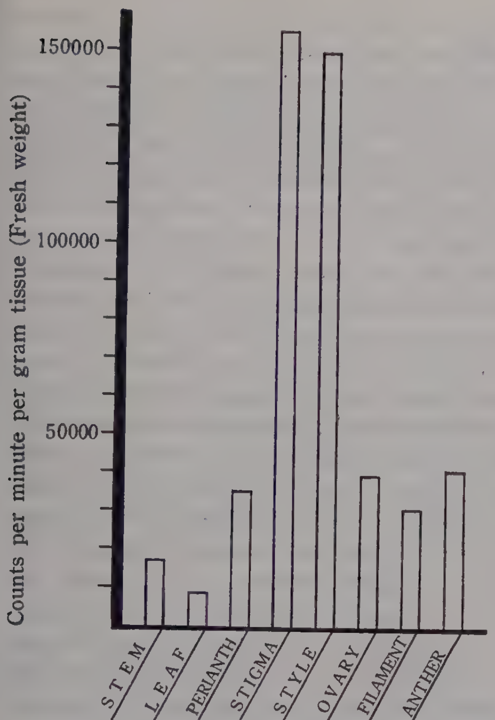


Fig. 2. Concentration of administered Co^{60} in various tissues of the mature flower bud.

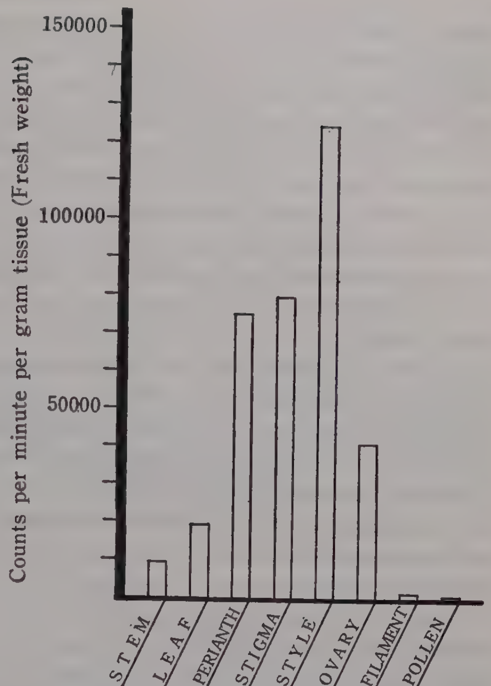


Fig. 3. Concentration of administered Co^{60} in various tissues of the open flower.

of Co^{60} were detected in the pollen grains. The stigma of open flowers showed much lower concentration of Co^{60} than mature flower buds. Excepting the style and perianth, such a decline in the distribution of Co^{60} was observed in other tissues which is concomitant with the development of floral tissues. The concentration of this element in the style was about 1.5 times that in the next highest tissue (the stigma) and about 6 times that in the leaf. Thus, the Co^{60} acquisition in the style remained almost unchanged in open flowers.

Discussion

The ability of certain plants to accumulate relatively large quantities of cobalt in their tissues has been reported by several workers[2,3,4]. Such an accumulation of cobalt in plant tissues has been of particular concern to agronomists because of the immense economic implications. However, very little information has so far been available concerning the acquisition of cobalt in various floral tissues. In the present study, the fate of radio-active cobalt in various floral tissues of *Lilium longiflorum*, from flower buds of various lengths, has been studied as a guide to the actual distribution of this element in tissues.

From the data obtained, it has been found that high concentration of Co^{60} accumulated in both stigma and style, but the degree of acquisition of this element in these two tissues was strikingly varied to the aging of flower. Thus, in young flower buds there was a marked acquisition of radio-active cobalt in the stigma, and to a lesser extent, in the style. However, the concentration of Co^{60} in the style and

other parts of the flower, ovary and filament, was similar to that in the leaf and stem. Moreover, Co^{60} was not concentrated in the anther including pollen grains to any appreciable extent in young flower buds. In the case of mature flower buds, there was a significant increase in the Co^{60} acquisition of the style. It was found that the highest concentration of Co^{60} accumulated in both stigma and style, and that the acquisition of Co^{60} was restricted about to two tissues. The reason for the differential acquisition of this element in the tissues is not known, but this fact may be connected in some way with the aging of the tissues. In the present experiment, the ovary, anther, filament and perianth showed the same degree of acquisition. The acquisition of Co^{60} in both leaf and stem was less significant than that in any other floral tissues. The changes in the Co^{60} acquisition during the flower development, however, were also observed in the anther. In the anther including pollen grains, the acquisition of Co^{60} was of a higher level in the mature flower bud than that in the young one. As differentiating of the flower bud, there occurred apparently a differential acquisition of Co^{60} in the style. In the case of open flowers of which the anthers had already dehisced, there was a higher acquisition in the style, a high in the stigma and perianth, and a low in the ovary. To emphasize the significance of the data, the ratio of the concentration of Co^{60} in the floral tissues to that in the leaf has been calculated. The ratio was 4 in the stigma, while 6.2 in the style. In other floral tissues, except the perianth, it never amounted to more than 2. Though Co^{60} was not greatly concentrated in the pollen grains and filament, a considerable quantity was found in the ovary. An acquisition of Co^{60} in the perianth did not appear to be important, as the amount of this element in the perianth was almost proportional to the development of flower bud.

From these observations, it may be concluded that one of the characteristics of style of the open flowers is its highly differential acquisition of the Co^{60}, although it is not possible as yet to exclude the possible acquisition of other radio-active isotopes in the tissue. It is difficult at the present time to know what factor or factors are responsible for this difference between the style and other floral tissues. However, the concentration of Co^{60} in the style is high enough to suggest that this element might play a significant rôle in the physiological function of this tissue. Whether this differential acquisition of the Co^{60} in the style is related to a specific function of the style or cobalt exists in a certain component of the tissue would become clear only after future investigation into its rôle in the stylar metabolism, and further experiments designed to approach these points are in progress.

Summary

The fate of radio-active cobalt (Co^{60}) in various floral tissues of *Lilium longiflorum*, from flower buds of various lengths, has been studied as a guide to the actual distribution of this element in tissues. As differentiating of the flower bud, there was a significant increase in the degree of acquisition of Co^{60} in the style. It was demonstrated that this radio-active isotope of the element is markedly concentrated in the style of open flower of which the anthers had already dehisced. This acquisition of Co^{60} in the style is high enough to suggest that this element might play a significant rôle in the physiological function of this tissue.

The writer is indebted to Dr. K. Imai, Institute of Endocrinology, Gunma University, and Dr. H. Suzuki, Tokyo University of Education, for their invaluable advice and criticism.

References

1) Yamada, Y., Bot. Mag. Tokyo **71**: 319 (1958). 2) Beeson, K. C., Gray, L., and Adams, M. B., Jour. Amer. Soc. Agron. **39**: 356 (1947). 3) ——, Lazar, V. A., and Boyce, S. G., Ecol. **36**: 155 (1955). 4) Yamagata, N., and Murakami, Y., Nature **181**: 1808 (1958).

摘　　　要

山田　義男: 花粉の生長におよぼすコバルトの効果 II テッポウユリの花柱組織による Co^{60} の特異的とりこみについて

テッポウユリ (*Lilium longiflorum*) の花の各組織に存在するコバルトの分布を予測するために，蕾の発育過程における各組織による Co^{60} のとりこみをしらべた．蕾が分化するにつれ，花柱による Co^{60} のとりこみの程度は急激に増加した．特に開葯した花の花柱は他の組織にくらべ，いちじるしく高濃度の Co^{60} をとりこむ．花柱におけるこのような Co^{60} の特異的とりこみは組織内の生理的機能に関し，おそらくコバルトが重要な役割をはたすものと推定される．(群馬大学学芸学部生物学教室)

Bot. Mag. Tokyo **73**: 422–426 (October 25, 1960)

ハッカの春化処理の発育および精油含量におよぼす影響*

大橋　裕**・市川郁雄***

Hiromu OHASHI, and Ikuo ICHIKAWA: On the Effect of Vernalization on the Development and the Content of Essential Oil of Pepermint-plant.

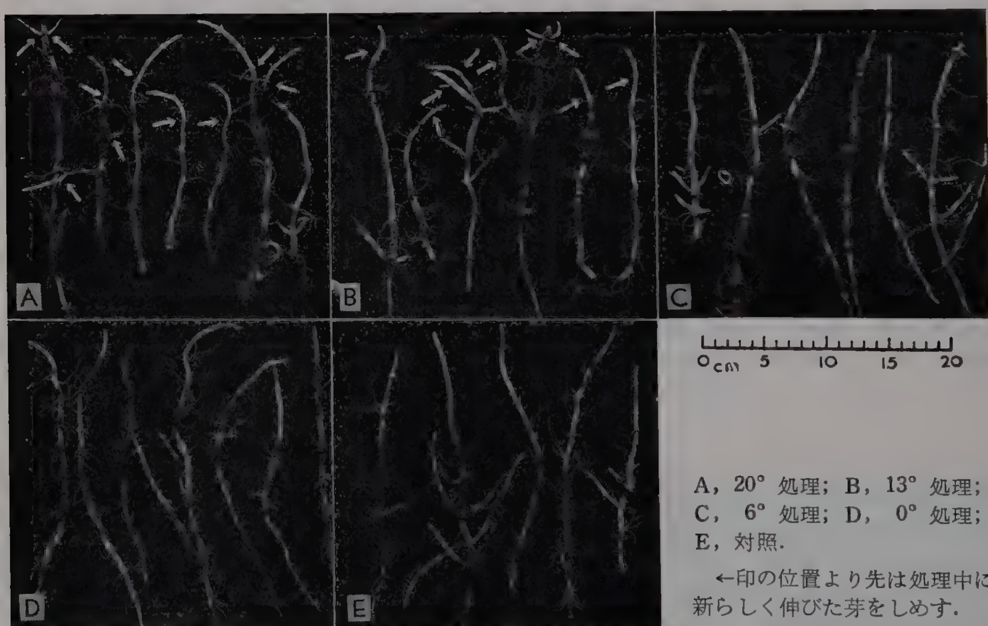
1960 年 3 月 3 日受付

従来ハッカ *Mentha arvensis* L. var. *piperascens* Holmes の春化処理にかんしておこなわれた実験はみあたらないようである．私たちは，ハッカをいろいろのことなった温度で処理し，これの発育や精油含量におよぼす影響にかんして，2, 3 の知見をえたので，ここに報告する．この実験は 1958 年に長崎大学附属薬草園（長崎市）においておこなった．

材料および方法

ハッカの繁殖法には，根植法（分根法），苗植法，挿木法，圧条法[1]などがある．通常もちいられるのは根植法で，これは 11 月上旬～12 月下旬に，地下茎を圃場に植付ける方法である．よって，ハッカの春化に関する本実験では，地下茎をもちいた．使用品種は赤茎種である．

1 回の処理に，ほりとったばかりの地下茎 600 g（径，約 0.5 cm; 長さ 10～45 cm; 約 200 本）をもちいた．これを処理中の乾燥をふせぐために，湿らせたサラシの布でつつみ，うすいポリエチレン製の袋におさめた．さらに処理中にときどき地下茎を



A, 20° 処理; B, 13° 処理; C, 6° 処理; D, 0° 処理; E, 対照.

←印の位置より先は処理中に新らしく伸びた芽をしめす.

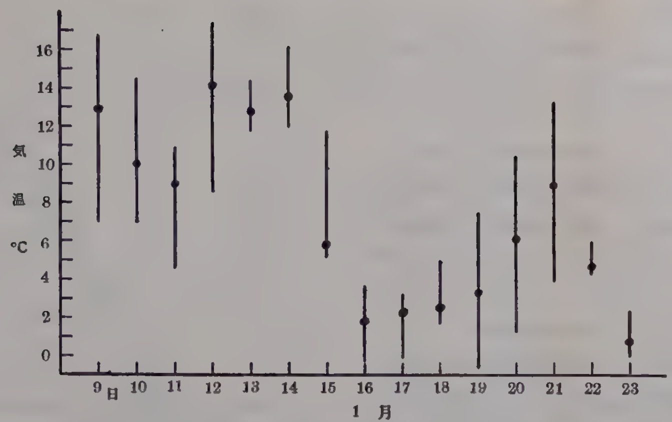
第 1 図　春化終了時の地下茎の状態

---

* 松浦　一・山田幸男両教授還暦記念論文.（薬用および油料植物の春化処理　第 9 報）.

** 長崎大学薬学部生薬学教室　Institute of Pharmacognosy, Faculty of Pharmacy, Nagasaki University, Nagasaki, Japan.

*** 長崎市中央保健所試験室　Service Section, Nagasaki Municipal Health Center, Nagasaki, Japan.



第 2 図　長崎市の日最高，平均，最低気温
（長崎海洋気象台）

つつんだ布をしめらせるとともに，地下茎に直接水を散布して乾燥をふせいだ．

春化は約 20°（20.2 ± 0.0°），13°（12.9 ± 0.2°），6°（5.6 ± 0.7°），および 0°（0.0 ± 0.8°）で，処理日数はながすぎると醗酵状態になるため，15 日間（1 月 8 日〜23 日）処理した．

処理がおわったときの地下茎の状態は，高温で処理した地下茎は発芽し，芽の長さは 20° 処理では 3〜6 cm，13° 処理では 2〜3 cm にたっしていたが，6° 処理，0° 処理，および対照の発芽はみとめられなかった（第 1 図）．

対照は，圃場にまとめて埋めておいたものをもちいた．参考のために，この間の長崎市の気温を第 2 図にあげた．

植付けは，春化終了直後の 1 月 23 日に，条間 60 cm で，ほぼ 1 本ならびにおこなった．肥料は，1a あたり堆肥 70 kg，硫安 2.3 kg，過燐酸石灰 1.5 kg，塩化カリ 0.8 kg をほどこした．

発芽後，株間約 15 cm に間引いた．この結果 20° 処理をのぞく他の処理や対照では，ほぼ等間隔で 40〜50 本を栽培することができた．しかし 20° 処理は発芽数がすくなかったので（後述），25 本を栽培しえたにすぎず，その株間も不揃いで 15 cm 以上になったところが多い．

結　　果

I．発　芽

20° 処理および 13° 処理の発芽，すなわち芽の地表にあらわれた日は，他の処理，対照に比してはやめられた．ところが各処理区の発芽数は春化によりいずれも対照より低下し，とくに 20° 処理においてはいちじるしかった（第 1 表）．

II．草丈の伸長

春化が生長におよぼす影響をしるために，5 月下旬から 8 月中旬にかけて，約 10 日おきに計 9 回草丈を測定し，平均値を求めた（第 3 図）．

20° 処理は発芽が促進されたにもかかわらず，その後の草丈の伸長はめだってわるく，つねに他の処

第 1 表　発　芽

処　　理	20° 処理	13° 処理	6° 処理	0° 処理	対　照
発芽始　月・日	II，26	II，26	II，26	II，26	II，26
平均発芽日　月・日	III，3.7 ± 2.18	III，4.5 ± 0.96	III，7.4 ± 0.82	III，8.2 ± 0.77	III，7.9 ± 0.6
同促進日数	4.2**	3.4**	0.5	−0.3	
発芽揃　月・日	III，18	III，20	III，19	III，20	III，19
発芽総数	44	163	170	171	217
同比率	20	75	78	79	100

発芽始，始めて発芽をみた日；平均発芽日，それぞれの芽が発芽した日の平均値；発芽揃，発芽数が最高にたっした日；** 対照にたいし危険率 1% で有意差あり．

理や対照より低い値をしめした．それに比して，13° 処理，6° 処理はつねに対照よりいくらか高い値をしめした．0° 処理は対照とかわらなかった．

Ⅲ. 開　花

春化が開花状態におよぼす効果は第2表にみられるように，処理温度と密接な関係があり，処理温度が高くなるにともなって，開花がはやまる傾向がみられた．とくに，20° および 13° 処理の開花は対照よりはやまった．

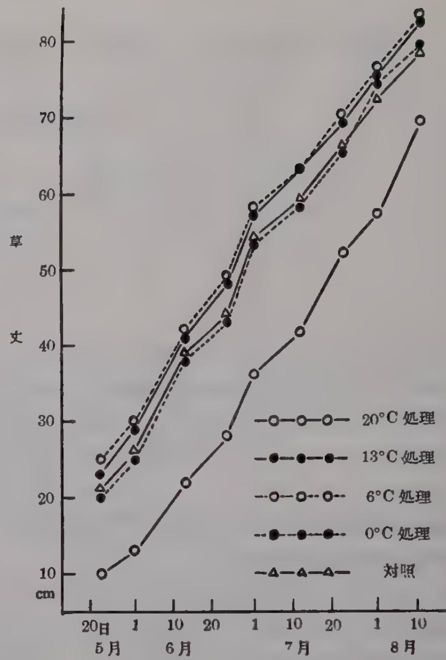
Ⅳ. 薬収量および 2，3 の形態的特性

9月4日に各処理，対照植物を同時に収穫風乾した後，草丈，主茎の節数，地上部重，薬重を調査した（第3表）．

20° 処理の草丈（13° 処理，6° 処理にたいして危険率 5%，対照にたいして同 10% で有意差あり），節数，地上部風乾重，薬風乾重は低い値をしめした．他の処理，対照間には有意差はみとめられない．

Ⅴ. 精油およびメントール含量

春化が有用成分の含量や質におよぼす影響をあきらかにするために，薬中の精油（ハッカ油）含量および精油中のメントール (*l*-menthol) 含量を定量した．



第3図　草丈の伸長

第2表　開　花

処　理	20° 処理	13° 処理	6° 処理	0° 処理	対　照
開花始　月・日	Ⅶ，26	Ⅶ，25	Ⅶ，28	Ⅶ，28	Ⅶ，25
平均開花日　月・日	Ⅷ，3.9 ± 3.55	Ⅷ，4.8 ± 2.44	Ⅷ，6.2 ± 2.69	Ⅷ，11.4±3.17	Ⅷ，8.4 ± 2.91
同促進日数	4.5*	3.6*	2.2	−3.0	
開花揃　月・日	Ⅷ，24	Ⅷ，28	Ⅷ，30	Ⅷ，28	Ⅷ，27

開花始，始めて開花をみた日；平均開花日，各株が開花した日の平均値；開花揃，全株開花した日；
* 対照にたいし危険率 5% で有意差あり．

第3表　薬収量および 2，3 の形態的特性（9月4日収穫）

処　理	20° 処理	13° 処理	6° 処理	0° 処理	対　照
草　丈　cm	90 ± 5.2	98 ± 3.4	98 ± 3.8	94 ± 3.0	95 ± 3.0
節　数	35 ± 1.5**	40 ± 1.2	39 ± 0.9	39 ± 1.0	39 ± 0.7
地上部風乾重 g	46 ± 7.3**	64 ± 8.5	66 ± 9.2	60 ± 8.0	58 ± 6.7
薬風乾重 g	10.6 ± 1.67**	17.3 ± 2.38	17.6 ± 2.84	16.3 ± 2.34	15.5 ∓ 1.75

** 対照にたいし危険率 1% で有意差あり．

薬中の精油含量の定量には，日本薬局方規定の精油定量器（比重1以下）[2] をもちい，1回に 40.0g の資料を採取し，それぞれ3回づつ定量した．この測定値は容量でえられるので，この値に比重 0.9 をかけて重量を算出した[3]．薬中の精油含量は，処理温度が高くなるにともなって増加する．対照の含量は

第 4 表　精油およびメントール含量

処　　理	20° 処理	13° 処理	6° 処理	0° 処理	対　　照
葉中精油含量 %	4.70±0.378**	4.36 ± 0.172	4.13 ± 0.168	3.99 ± 0.490*	4.26 ± 0.202
同　比　率	110	102	97	94	100
精油中メントール含量%	79.97	80.93	81.63	81.68	81.57
同　比　率	98	99	100	100	100
葉中メントール含量 %	3.76	3.53	3.37	3.26	3.47
同　比　率	108	102	97	94	100

** 対照にたいし危険率 1%, * おなじく 5% で有意差あり.

ほぼ 13° 処理にひとしい.

精油中のメントール含量の定量は，精油の定量とは別に，水蒸気蒸溜により多量に抽出，脱水した油をもちいて，清水氏法[4] によりおこなった．精油中のメントール含量の変化はほとんどなく，わずかに高温処理において低下した．そのため，葉中のメントール含量は，精油含量の変化に対応し，処理温度がたかいほど増加する傾向がみられる (第 4 表).

考　　察

高温 (20°, 13°) 処理における発芽の促進は，春化により，これらの地下茎が植付時にすでに発芽していたことより，当然の結果であると考えられる．春化にともなう発芽数低下の原因はよくわからないけれども，(1) 外観上の変化はみとめられないが (第 1 図)，処理中不自然な状態におかれたことによる地下茎の乾燥，(2) とくに 20° 処理がわるかったのは，高温でいちじるしく長く伸びた芽が，急に冬期の低温下の圃場に植付けられたことによるのではないかと思われる．ハッカの地下茎は，多量の水分 (約 80%) を含有しており，通常の栽培においても乾燥した地下茎を植付けると発芽率が低下することがしられている[1]).

20° および 13° 処理において，開花の促進がみとめられたことより，ハッカは 13°～20° ないし，それ以上の高温で，その温度発育段階 (vernalization phase)[5]) を通過すると考えられる．

Crocker and Carton[6])によると，秋まき性植物は 0°～10°，春まき性植物は 20°～30° の温度を，この段階の通過にさいして要求し，私たちの研究[7]) においても，ほぼ同様な結果がえられ，前者は 0°～10°，後者は 15°～35° を要求する．

ところが，ハッカは地下茎が晩秋ないし初冬に植付けられ，この時期よりすると，秋まき性植物であると考えられるが，温度発育段階の通過にさいして春まき性植物のような高温を要求し，従来の例からすると，矛盾した結果をしめす．植物の播性は，それが播種または植付けた時期により判断するより，発育にさいして要求する環境諸条件 (とくに温度，光条件) より決定すべきであると考えられる．かかる観点よりすると，すくなくともハッカは温度発育段階の通過にさいして高温を要求し，むしろ春まき性植物であるとするのが妥当であると思われる．ハッカにおいて，かかる矛盾の生じた理由は，一般の秋まき性植物は秋期播種されると，ただちに発育を開始して，発芽し地上部を形成し，冬期の低温を感温しながら越冬するが，秋に植付けられたハッカの地下茎は翌春まで発芽しなくて，この間，地下茎は冬眠状態にあり，温度発育段階を進行しないことによると考えられる．

春化により成分含量の変化がみとめられたが，この主要な原因は，(1) 春化による発育の変化にともなう相対的変化，(2) 春化の直接的効果，によると考えられる．

ハッカ葉中の精油含量および精油中のメントール含量は，ともに発育と密接な関係があり，発育がすすむにともなって増加することがしられている[8,9]) (ただし精油含量は発育の末期にいくぶん低下する).

春化が精油含量および質におよぼす効果を観察してみると，精油含量の変化は，処理による発育の変化状態とよく一致しており，発育が促進せられる高温処理ほど高い．ところが，精油中のメントール含量はほとんど変化なく，かえって高温処理ほど低下する傾向すらみとめられる．

これらのことより，春化による成分の変化は，(1) の影響より，むしろ，(2) の原因により，処理温度

の直接的効果に，より大きく支配された現象ではないかと考えられる．なお，この問題にかんする考察は第6報[10])にくわしい．

要　　約

ハッカの地下茎を約 20°，13°，6°，0° の4段階の温度で，それぞれ 15 日間処理した．対照としては，この間土中に埋めておいたものをもちいた．

1. 20° 処理，13° 処理の発芽は他の処理，対照よりはやまった．しかし，春化をおこなった処理の発芽率は低下し，とくに 20° 処理ではいちじるしかった．

2. 20° および 13° 処理の開花は対照よりはやまった．よって，ハッカは 13°～20° ないしそれ以上の高温で温度発育段階を通過し，春まき型の植物であると考えられる．

3. 20° 処理は，発芽のみならず，その後の生長も不良で，収穫時の草丈，地上部重，葉収量も低下した．他の処理，対照間にはあきらかな差はみとめられない．

4. 葉中の精油含量は処理温度が高くなるにともなって増加した．対照の含量はほぼ 13° 処理にひとしい．精油中のメントール含量はほとんど変化しない．これらの変化は，主として処理温度の直接効果にもとずくと考えられる．

本研究にあたり御校閲をいただいた北海道大学理学部松浦一，宇佐美正一郎両教授に深謝する．メントールの定量にかんする貴重な文献をいただいた信州大学理学部清水純夫氏にたいして深謝する．また栽培管理を熱心におこなってくださった長崎大学薬学部南里卯吉氏に感謝する．

文　　献

1) 井上弘，(佐々木喬監修) 綜合作物学，工芸作物篇，嗜好料の部薬用の部，東京，186 (1953). 2) 第6改正日本薬局方註解，東京，1173 (1959). 3) 福島要一，日作紀 **11**: 147 (1939). 4) 清水純夫，信州大学農学部紀要 **4**: 62 (1954). 5) Lysenko, T. D., Agrobiology, Moscow, (1953). 6) Crocker, W., and Barton, L. V., Physiology of Seeds, Waltham, 205 (1957). 7) 大橋　裕，アグロビオロギヤ No. 3 (123): 427 (1960). 8) 三宅康次・石塚喜明，土肥誌 **12**: 376 (1938). 9) 大橋　裕・市川郁雄，生科 **11**: 38 (1559). 10) 大橋　裕・市川郁雄，植雑 **73**: 239 (1960).

Summary

Rhizomes of pepermint-plant (*Mentha arvensis* L. var. *piperascens* Holmes) were vernalized at four temperatures, 20°, 13°, 6° and 0°, for 15 days. The rhizomes buried in the ground during the period of temperature treatment were used as control.

(1) The 20° and 13° treated groups sprouted earlier than those of the other treatments and the control. But the sprouting rate of the vernalized groups was lower than the control, especially in the case of 20°.

(2) The flowering season was investigated to clarify the effect of the vernalization upon the development. The 20° and 13° treated groups flowered about 4 days earlier than the control. The 6° and 0° treated ones, however, showed no significant difference in flowering season to the control. Therefore the pepermint-plant seems to pass through its vernalization phase at comparatively high temperature, at least from 13° to 20°. The pepermint-plant is presumed to be rather a spring form in spite of the rhizomes of this plant being planted in late autumn to early winter for its propagation.

(3) There were no significant differences between the treated groups (except 20° treated one) and the control in the height of plant, weight of stem and leaf, and leaf crop at the harvest time. The 20° treated group showed minimum growth.

(4) The average content of essential oil in leaves increased in proportion to the rise of temperature of treatment, and the content of the control was equal to that of the 15° treated group. It is conceivable that this variation was produced mainly by the direct effect of temperatures of treatment. The relative content of menthol in essential oil did not show any significant difference among the treated groups and control.

Bot. Mag. Tokyo **73**: 427-430 (October 25, 1960)

セキショウ成分の生理化学的研究

第5報　アミノ酸および糖

遠藤庄三*・丹治一義*

Shōzō ENDŌ and Kazuyoshi TANJI: Physiological Chemistry of *Acorus gramineus* Soland. V. Studies on Amino Acids and Sugar Components.

1960年3月15日受付

緒　言

セキショウはわが国の山野，陰湿地に野生する多年草本であって広く庭園に栽培されているが，特に静岡，茨城に多い．

その成分についての研究はすくなく，木村[1]が根茎を水蒸気蒸溜し，パルミチン酸，アザロン（4-プロペニル-1,2,5-トリメトキシベンゼン）および未詳のフェノール性物質を報告したにすぎない．

筆者らは，これまで顕微化学的に各器官の成分を明らかにし，またペーパー・パーチションクロマトグラフィ（以下 P.P.C. と略す）によって成分を検討してきた．前報[2]において冬期に多量の結晶性炭水化物の存在することを確かめたが，この物質の同定およびアミノ酸の単離について報告する．

実験の部

I　試料の調整

静岡県庵原郡小島村に自生するセキショウを2月中旬採集して，地下茎のみを用いた．

風乾細切試料 6kg に水 6*l* を加え，ときどき攪拌しながら一昼夜放置した後，圧搾抽出し，残渣を再び同様に処理して褐色液 10*l* を得た．これに活性炭 200g を投入し 80° で15分加熱後ブフナー漏斗上に活性炭の薄層を作って通過させれば無色透明液となる．沪液はアンバーライト IR-120（R-H 型）にて塩基を，また IRA-400（R-OH 型）にて酸を捕集し，沪液を中性成分とした．

* Biological Institute, Faculty of Education, Shizuoka University, Shizuoka, Japan.　静岡大学教育学部生物学教室

II　塩基性成分の分離

A　アスパラギンの分離

アンバーライト IR-120（5cm×40cm，3cm×30cm を直列につなぐ）に捕集された塩基を 0.15N アンモニアにて置換溶出し 50m*l* のフラクションに分取した．各フラクションを P.P.C. にて検討した結果は常時 4～5 種のアミノ酸が混在し，イオン交換クロマトグラフィとしての意義も薄いので全フラクションを合して減圧濃縮し，一夜氷室に放置すれば結晶が析出する．3日の後吸引沪取し，水より3回再結晶すれば無色透明斜方柱状晶となる．P.P.C. によりなお微量の夾雑物を認めるので，さらに3回再結晶を繰返す．m.p. 225°，冷水に難溶，エーテル，アルコールに不溶，ビューレット反応陽性，標準試薬アスパラギンと対照して P.P.C. を行なったが差異を認めなかった．

分析結果:

実験値　C 32.03，H 6.68，N 18.73%

$C_4H_8O_3N_2 \cdot H_2O$ としての理論値

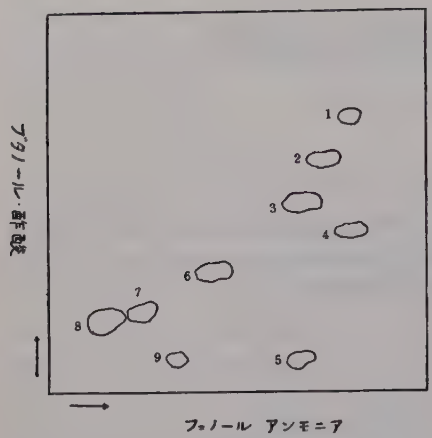
C 32.00，H 6.71，N 18.66%

結晶を硫酸浴中で加熱すれば 105～110° で結晶水を失って，白色不透明となる．

B　セルロースカラムクロマトグラフィ

アスパラギンを分離した母液にはなお第1図に示すように9種のアミノ酸が混在する（展開剤，1次元: ブタノール/酢酸/水＝4: 1: 5，2次元: フェノール/$^1/_{10}$N アンモニア＝9: 1)[3]．スポットはいずれも試薬と対比して同定したがプロリンのみは色調および相対的位置より判定した．

つぎにセルロース 100g，ブタノール/酢酸/水（4: 1: 5）を用いて湿式法により作成した 3cm×60cm のカラムに，試料 5m*l* を溶媒に溶解して静かに注



1，イソロイシン 2，バリン 3，γ-アミノ酪酸 4，プロリン 5，ヒスチジン 6，アラニン 7，グリシン 8，アスパラギン酸 9，アスパラギン

第1図 アミノ酸の二次元 P.P.C.

入し，一様に浸透の後展開し，流出液を 10 ml づつ分取した結果は第2図の通りであった．

フラクションAは第1図では認められなかったがこのものはニンヒドリンによる色調が淡く微量の試料を2次元展開したためと考える．

単一フラクションのみを合して減圧濃縮し，残渣を再結晶した結果，B，C，G の各フラクションより微量の結晶を分離した．

i) イソロイシンの結晶化

スポットB部分（フラクション 8～12）を集めて減圧濃縮すれば淡黄色粉末となる．少量の水に溶解し倍量のメタノールを加えて沈澱物を除き，さらに等量のエタノールを加えれば，白色結晶性粉末となる．粉末を 70% アルコールより3回再結晶すれば m.p. 272～274° の微細結晶となる．イソロイシンと混融しても融点降下を示さない．

ii) バリンの結晶化

スポットC部分の各フラクションを合して減圧濃縮し，残渣を熱メタノールに溶解し，3倍量のアルコールを加えて放冷すれば微細箒状結晶が析出する．さらに1回同様に処理して不純物を除いた後，80% アルコールより3回再結晶を繰返せば m.p. 295° となりバリンと混融しても融点降下を認めない．

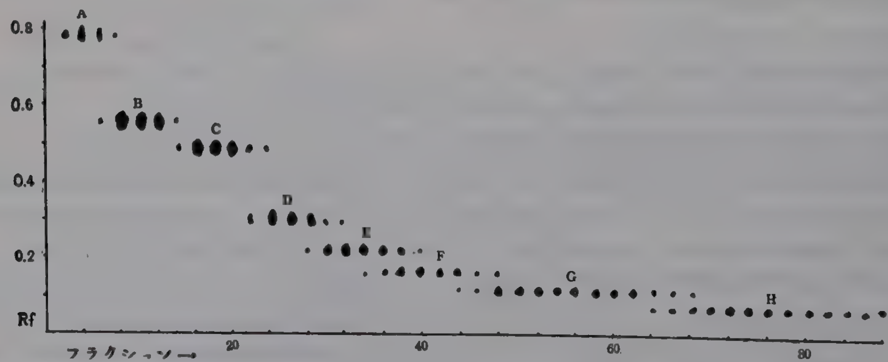
iii) アラニンの結晶化

スポットG部分を減圧濃縮し，熱メタノールに溶解せしめ活性炭で脱色の後エタノールを加えて結晶せしめる．90% エタノールより2回再結晶すれば m.p. 289～292°，アラニン（m.p. 294～295°）と混融すれば m.p. 291～293° となる．

以上のアミノ酸はいずれも微量のため元素分析することができなかった．P.P.C. で検討したが試薬との差異を認めなかった．

III γ-アミノ酪酸の単離

以上の結果はいずれも微量のため元素分析に至らなかった．そこで新たに試料 4 kg を用いて抽出操作を繰返し陽イオン交換樹脂よりの溶離液を便宜上2分して捕集した結果，前半部より γ-アミノ酪酸を分離した．濃縮残渣に等量のアルコールを加えれば直ちに二層を形成し，冷蔵放置すれば下層よりア



A，未同定；B，イソロイシン；C，バリン；D，γ-アミノ酪酸；E，プロリン；F，アラニン；G，アスパラギン＋グリシン；H，ヒスチジン＋アスパラギン

第2図 セルロースカラムクロマトグラフィによるアミノ酸の分離状態

スパラギンが析出する．上層液を傾斜してとり濃縮してシラップとし，熱メタノールに溶解して不溶物を除き放冷すれば結晶が析出する．結晶を沪取し80%アルコールより4回再結晶すれば無色板状晶となる．m.p. 191°

分析結果：

実験値 C 46.03, H 8.80, N 13.53%

$C_4H_8O_2N$ としての理論値

C 46.59, H 8.80, N 13.58%

アミノ酪酸に相当する．P.P.C. の結果および融点から γ-アミノ酪酸と確認した．

IV 中性成分の分離

A マンニットの確認

前報[2]に報告した物質がマンニットであることを確認した．すなわち，試料を陰陽交換樹脂にて処理して得た沪液 12l を 58° 以下の浴温で減圧濃縮し淡黄色シラップとなし，倍量のエタノールを加えて1週間放置すれば，マンニットが箒状となって析出する．結晶をメタノールより5回再結晶すれば稜柱晶，m.p. 165° となる．母液はさらにエタノールを加えて結晶の析出を促がし乾燥後秤量すれば 180.5g, 供試材料の約3% に相当する．P.P.C. の結果は試薬マンニットと差異を認めない．

i) 分子量の測定（氷点降下法，溶剤：水）

試料	0.999	1.854	2.733g;
$\Delta T =$	0.333	0.6243	0.940.
実験値 分子量	185.6	184.1	182.9,
		平均	184.2
$C_6H_{14}O_6$ としての理論値			182.9

ii) アセチル化

無水の酢酸ナトリウムおよび無水酢酸で常法によりアセチル化した後，氷水中に加えて一夜放置すればアセタートが析出する．稀酢酸より5回再結晶すれば m.p. 122° となる．

分析結果：

実験値（平均） C 49.585, H 6.125%

$C_{16}H_{24}O_{11}$ としての理論値

C 49.0, H 6.10%

iii) アセタートの分子量(氷点降下法，溶剤：水)

試料 0.825, 1.780g, $\Delta T = 0.25, 0.65.$

実験値 分子量 393.1, 392.9, 平均 393.0

$C_6H_9O_6(CH_3CO)_5$ としての理論値 392.0

iv) アセチル基の分析結果

試料 3.710mg, 1/10N NaOH 消費量 3.94ml,

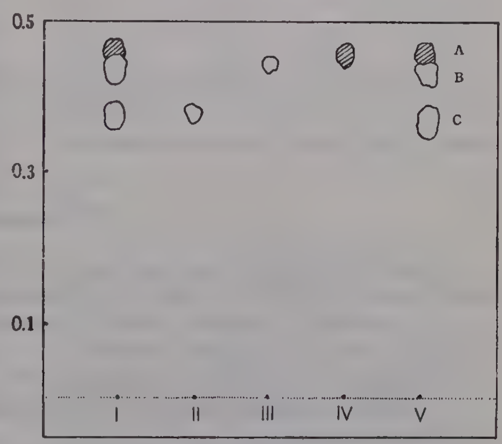
アセチル 51.93%

$C_6H_9O_6(CH_3CO)_5$ としての理論値 54.84%

マンニットを除去した母液は減圧濃縮してアルコールを除き糖の検索に供した．

B P.P.C. による糖の定性

試料および試薬を沪紙（東洋沪紙 No. 50, 40cm×40cm）の一端より 4cm の線上に 3cm の間隔に添着し，n-ブタノール/酢酸/水（4: 1: 5）を用いて3回反復上昇展開した．結果は第3図に示す通りである．フタル酸-アニリンを噴霧加熱すれば Rf 0.44, 0.38 の2つのスポットが検出され，塩酸-レゾルシンでは Rf 0.45～0.46 に顕著な桃赤色スポットが認められる．発色剤の特性および試薬との混合展開の結果より，前者グルコースおよびマンノースであり，後者はフルクトースであると考える．



I，試料; II，グルコース; III，マンノース; IV，フルクトース; V，混合

第3図 単糖類の P.P.C.

C グルコースの同定

試料 2g に純酢酸 20ml を数回に分けて攪拌しながら加え，最後に暫時加熱溶解の後放冷し3週間氷室に貯えれば微細箒状のグルコースが析出する．m.p. 146° 試薬と混融しても融点降下を示さない．また塩酸フェニルヒドラジンにて常法によりオサゾンを生成し，60% アルコールより5回再結晶すれば m.p. 204° となる．

分析結果：

実験値 C 60.16, H 6.19, N 15.65%

$C_{18}H_{22}O_4N_4$ としての理論値

C 60.32, H 6.19, N 15.63%

D マンノースの同定

試料 1g に塩酸フェニルヒドラジン 2.2g, 酢酸ナトリウム 3.3g を加えて攪拌溶解して放置すればマンノースフェニルヒドラゾンが析出する. 3時間の後沪取し, 水, 無水エタノール, 無水エーテルで順次洗浄の後 60% アルコールより4回再結晶すれば無色光沢のある斜方状結晶となる. m.p. 185°, D-マンノースフェニルヒドラゾンと混融しても融点降下を示さない.

考 察

セキショウ根茎中の遊離アミノ酸9種を P.P.C. によって同定したが, いずれもタンパク質を構成する普通のアミノ酸であって特別な生理作用をもつとも考えられない.

マンニットを貯蔵物質として含むものに, モクセイ科, セリ科植物がしられている. 浅井[4]によればクチナシのマンニット含量の変化は温度の影響によるものであるが, 糖を十分に与えれば夏期でもマンニットを貯蔵することができ, 同植物のマンニットと澱粉の消長は対照的である. セキショウのマンニットの季節的消長もクチナシと同様で 5〜10 月にかけては再度の追試にもかかわらず結晶化することができなかった. これが温度条件の変化によるものか, また前駆物質がどのような糖に属するかは今後の研究により明らかにしたい. またマンノースの存在を認めたが, これは 11 月下旬に採集した材料についてであって, 2月に採集したものではまだ確認していない. このことは植物の代謝における過渡的生産物としてのみマンノースは遊離で存在するという B.S. Mayer[5] の考えと一致するが, 本実験のみでは明らかでない.

文 献

1) 木村雄四郎, 薬誌 **46**: 380 (1925). 2) 遠藤庄三他, 静岡大学教育学部研究報告 No. 7 137 (1956). 3) 小清水弘・清水哲夫, 農化 **30**: 63 (1956). 4) Asai, T., Jap. J. Bot. **6**: 63 (1932-3), **8**: 343 (1936-7). 5) Mayer, B. S., and Anderson, D. B., Plant Physiol. **34**: 372 (1959).

Summary

In this study, amino acids and sugars contained in the rhizome of *Acorus gramineus* Soland were investigated by the ion exchange method and cellulose column chromatography.

1. Asparagine, isoleucine, valine, γ-aminobutyric acid and D-glucose were isolated in crystalline state from aqueous extracts.

In addition, the presence of mannose and glucose was confirmed by their derivatives.

2. Proline, histidine, alanine, glycine, aspartic acid and fructose were detected by paper chromatography.

3. An unidentified substance which had been reported in a preceding paper[2] was determined as mannitol, and it was found that the mannitol in this plant is a reserve substance during winter.

ニガナ類の分化について—(1)

高山植物と海岸植物の雑種および二，三の問題

西　岡　泰　三*

Taizo NISHIOKA: Phylogenetic Studies in *Ixeris dentata* Group, 1. Hybridization between the Alpine and Seashore Plants and Some Other Observations.

1960 年 4 月 22 日受付

ここで取扱うニガナ類というのは，北村[1]の分類に従うと，キク科 (Compositae)，タンポポ亜科 (Cichorioideae)，ニガナ属 (*Ixeris*) の Ixeridium 節に含まれるニガナ (*Ixeris dentata*) およびその亜種変種など 8 種類程の植物を指している．

ニガナ類は日本では海岸から高山まで広く分布しているが欧米にはその分布をみないので Babcock, E. B. *et al.*[2])が論文に引用しているのみで欧米には具体的な研究例はない．染色体基本数は $x=7$ で，染色体数を基本としてニガナ類の本邦における分布状況を考察すると，高山には 2 倍体 ($2n=14$ *Ixeris dentata* subsp. *alpicola* タカネニガナ) と 4 倍体 ($2n=28$ *I. dentata* subsp. *Kimurana* クモマニガナ) が，普通の山野には 3 倍体 ($2n=21$ *I. dentata* ニガナ，など) が，また海岸 (裏日本海岸) には 2 倍体 ($2n=14$ *I. dentata* subsp. *nipponica* イソニガナ) の存在がみとめられる．これらのうちで 2 倍体のみが通常の両性生殖をおこない，3 倍体 4 倍体はともに単為生殖を常習とするのである．岡部[3])，竹本[4,5,6])，西岡[7])，北村[8])の資料に著者の資料を加えて，特殊環境に生育する高山植物と海岸植物の分布状況の概略を図示する (第 1 図)．ニガナ類の倍数系列の基本である 2 倍体が高山と裏日本の海岸にのみ見つけられることは興味ある問題である．竹本[6]) は低地に見出される海岸植物のイソニガナがニガナの原始型に近いのではないかと推測している．核型分析のみから事実を推論することはできないので，著者はさしあたり高山と海岸とに隔離されている 2 倍体植物間の関係を調べるため交配を試みたのである．本稿ではこの結果と，3 倍体の核型についての考察や新たに得られた高山植物の性質などについて概略的にのべる．

* Department of Biology, Faculty of Science, Tokyo Metropolitan University, Tokyo, Japan.
東京都立大学理学部生物学教室

材料と方法

材料はすべて原地で採集し東京の実験園に鉢植されている．学名の取扱は北村 (1956) によっている．

Ixeris dentata subsp. *alpicola* タカネニガナ，高山植物
- $2n=14$　南アルプス　仙丈岳産

Tanigawa-nigana タニガワニガナ (仮称) 高山植物
- $2n=14$　上越国境谷川岳産

I. dentata subsp. *nipponica* イソニガナ　海岸植物
- $2n=14$　新潟県柏崎産

I. dentata var. *albiflora* f. *amplifolia* オオバナニガナ (ハナニガナ)
- $2n=21$　八甲田山
- 奥多摩高水山
- 谷川岳山ろく
- 多摩丘陵

I. dentata subsp. *Kimurana* クモマニガナ　高山植物
- $2n=28$　八甲田山
- $2n=21?$　南アルプス　荒川岳産

交配材料として用いられている高山植物と海岸植物について少し説明する．

タカネニガナは本州の高山にある普通の高山植物である．雑種研究上対象となる外部形態の主な特徴はつぎのような点である．細くてあまり丈夫でない茎を 2，3 本立て小数の頭状花をつける．総苞内片

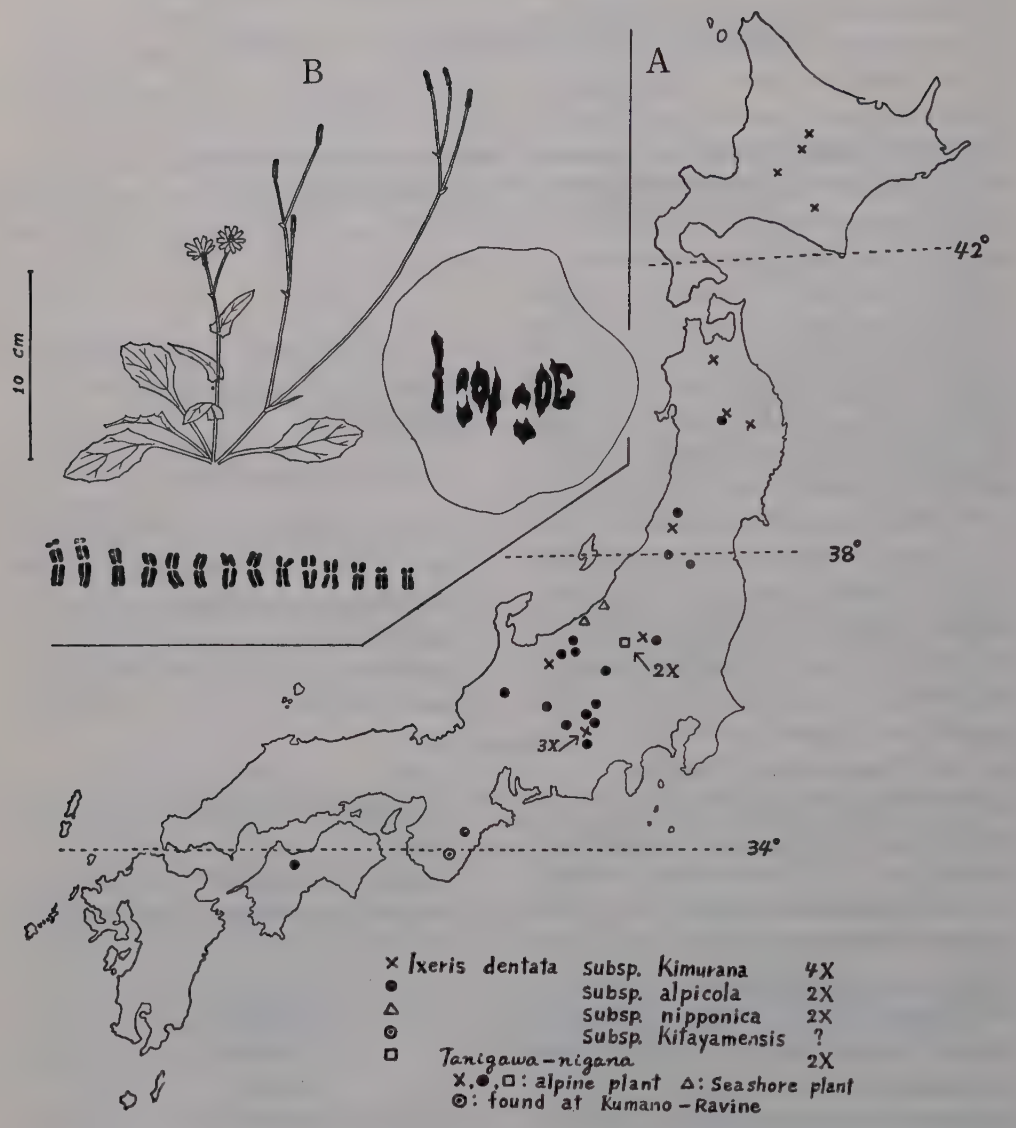


Fig. 1.

A. Distribution map of *Ixeris dentata* group, except the prevailing triploid plants.

B. "Tanigawa-nigana" and its chromosomes (ca. × 1000). This plant is sometimes bimorphic in the shape of stems: It has both upright and procumbent stems. $2n=14$, 7_{II}

Addendum

After sending the manuscript to press, *I. dentata* subsp. *kitayamensis* proved to be diploid.

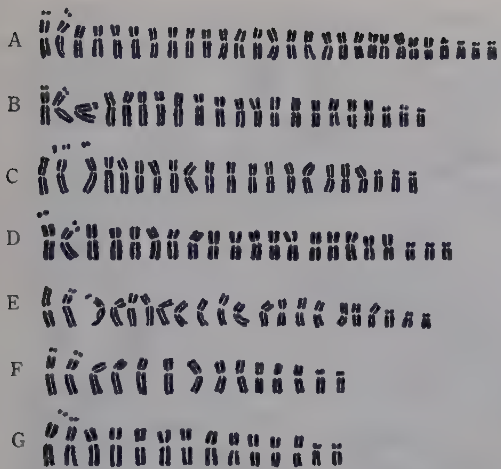


Fig. 2. Somatic chromosomes of some members of *I. dentata* group. ca. ×1000.
A: *I. dentata* subsp. *Kimurana* $2n=28$. B, C, D: *I. dentata* var. *albiflora* f. *amplifolia* $2n=21$ from the different localities respectively. E: Newly found triploid alpine plant $2n=21$. F: *I. dentata* subsp. *alpicola* $2n=14$. G: *I. dentata* subsp. *nipponica* $2n=14$.

8個小花数は 10 個，茎中部の葉は線状皮針形で基部では茎を抱かない．そう果にはやや長い嘴がある．体細胞染色体は仁染色2本を含み 14 本，減数分裂では7個の2価染色体をつくる． 3000m 付近の高地から低地に移植されるため栽培に困難を感ずるが，低地においても外部形態上の特徴は失なわれない (Plate 2. A, B).

谷川岳で著者がみつけた新しい型の2倍体植物に対してタニガワニガナと仮に名づけられている．体細胞染色体は 14 本，減数分裂では7個の2価染色体をつくる．この植物は，従来の2倍体の高山植物タカネニガナとはかなり違った外部形態を持っている．つまり茎は直立し太く，茎中部の葉は皮針形で基部は抱茎し，そう果も長い嘴を持たない． 3倍体のハナニガナの小型のものといった感じを受ける．この植物は面白いことに時折本来の茎の外にタカネニガナのそれを思わせる茎をだすのである．このことはハナニガナ型とタカネニガナ型の2つの特徴を合わせ持つことを意味している．しかしこの植物の葉はタカネニガナのように繊細ではない (Fig. 1. B).

イソニガナは海岸植物材料として使われた．この植物については竹本[6]によって分布や核型や自家不和合性などについて有意義な報告がなされた．14本の体細胞染色体を持ち減数分裂では7個の2価染色体をつくる．外部形態は前記の二者とは若干相異し葉はかなり厚く，茎中部の葉は丸みをおびていて基部では抱茎する．そう果には長い嘴はない (Plate 1. A, B).

交配は 1957 年と 1958 年の両年の5月に行なわれたが，1957年は開花時期のずれから成功しなかった．交配には従来の方法，つまり柱頭が開かない前に花柱についた花粉を強い水流で落し柱頭が開いてから受粉させる方法が用いられている．

得られた種子は 25° の恒温室におかれたペトリ皿中の吸水させたバーミキュライト上で発芽させる．交配の翌年に開花させるため，雑種植物は 20° の恒温箱中で 6000 ルクスの螢光燈全日照明下で2月末まで生育させる．後戸外のフレームに出して長日処理を与える．

体細胞染色体の観察には根端を8オキシキノリン前処理後酢酸オルセイン押しつぶし法で処理したものを，減数分裂の観察には葯をカルノア固定後酢酸カーミン押しつぶし法で処理したものをそれぞれ用いた．花粉の稔性は酢酸カーミン染色による花粉の内容充実度を判定の資料とした．

観　　察

1. 3倍体植物の核型について

3倍体植物の1種であるハナニガナの核型は産地によって異なっていて2倍体を構成する半数染色体組のそのままの3倍ではない．倍数性を端的に表わすのは，特徴のある形をもつ仁染色体と，最小の次末端狭窄染色体とである．各地で採集したハナニガナの核型を第2図に示してある．最小の染色体は比較的よく揃っているが，仁染色体の構成にはいくつかの型がある．同型のものが3本揃っているもの，3本あるが腕の比の異なるものが1本混じるもの，2本しかなく，その代りにそれらに見合う大きさの2次狭窄のない染色体が1本あるものなどである．竹本[4]は岡山産のものでは最大の仁染色体は1本であると報告している．八甲田大岳産のクモマニガナでは4倍体なのに仁染色体は2本しか観察されない．この結果は竹本[4]の結果と同様である．更に調査が必要であるが3倍体や4倍体における仁染色体の2次狭窄の消失現象はかなり一般的のものではないか

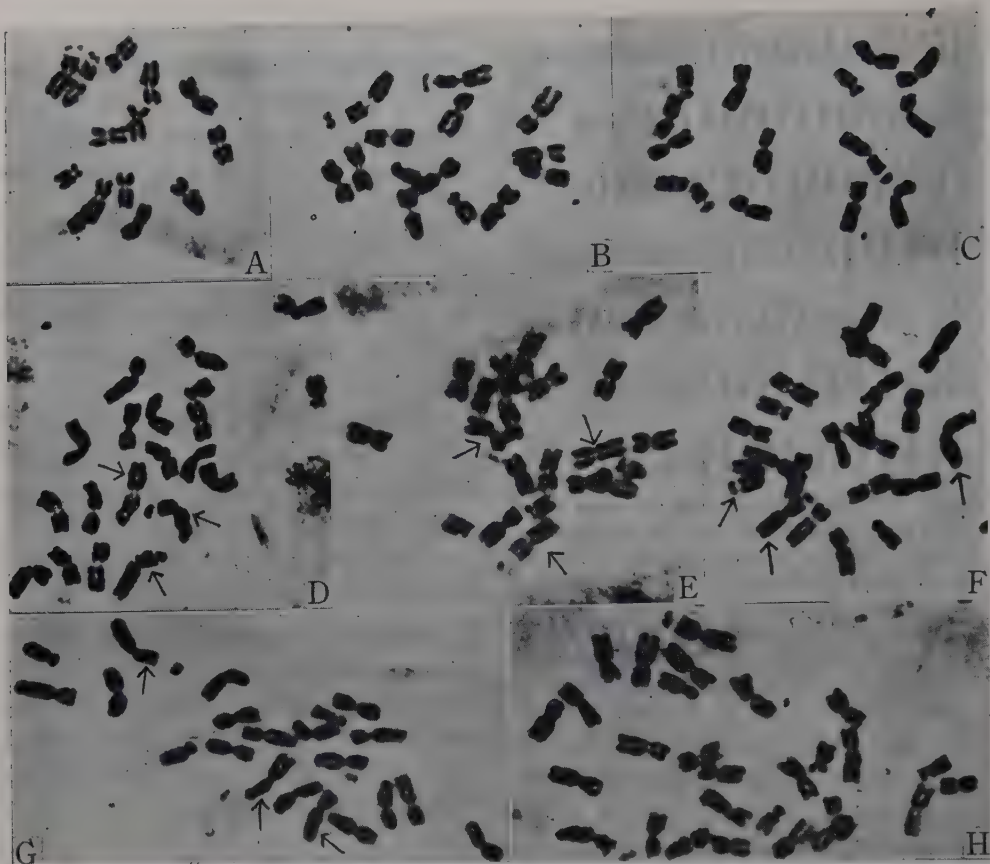


Fig. 3. Somatic chromosomes of "Tanigawa-nigana" (A), *I. dentata* subsp. *nipponica* (B), *I. dentata* subsp. *alpicola* (C), Newly found triploid alpine plant (D), *I. dentata* var. *albiflora* f. *amplifolia* (E, F, G) from the different localities respectively, and *I. dentata* subsp. *Kimurana* (H). Arrow indicates nucleolar chromosome and the equivalent one. ca. ×1600.

と思われる (Fig. 2. A, B, C, D., Fig. 3. E, F, G, H).

著者は 1959 年の夏南アルプス荒川岳でクモマニガナと思われる植物を採集した．この植物は 3 倍体であり，もし純然たる高山植物であるなら極めて興味深い植物である．高山に残されている 3 倍体があるのなら，それは 2 倍体のタカネニガナと 4 倍体のクモマニガナとの間を埋めるものではなかろうか．仁染色体は 2 本しか観察されない (Fig. 2. E, Fig, 3. D).

2．高山植物と海岸植物との交配

地理的隔離のみではなく，何らかの生理的隔離が高山植物と海岸植物の間に生じているかどうかを確かめるため交配が計画された．高山植物として 2 つの型つまりタカネニガナとタニガワニガナとが用いられた．

a．タカネニガナ×イソニガナ

開花時期のずれと悪い種子形成率が重なって得られた雑種種子はわずか 8 個であった．そのうち発芽したのは 7 個，2 個体は発根不良で枯死したため育ったのは 5 個体となった．翌年開花期になっても 5 個体のうち 1 個体はロゼット葉のままで残った．茎立ちし開花した雑種植物は 4 個体であったが，それらの外部形態は全体的にタカネニガナに近い感じで葉が厚くなったことや茎中部の葉が多少丸みをおびその基部がやや抱茎することなどにイソニガナの形質がみとめられる．雑種の体細胞染色体数はいずれの個体でも 14 本で，数の上の変化はみとめられな

いが，生存した5個体のうち2個体では，染色体の構造変化が起った．この構造変化は，仁染色体で明瞭にみとめられる．1対の仁染色体の一方の仁染色体では腕の長さに変化を生じ，他方の仁色体ではその2次狭窄が不明瞭化したのである．これらの変化のうち前者は，減数分裂の観察によって，仁染色体の腕の転座によるものであることが明らかになった．減数分裂で，1個体では4価染色体を，他の個体では4価染色体のほかに3価染色体や6価染色体を生じたのである．第2分裂以後の行動には異常はみとめられない．しかし花粉の稔性は極めて低く30%以下である．染色体異常のみとめられない雑種個体でも，減数分裂は正常なのに花粉の稔性は低くて45% 程度であった．結局，得られた雑種個体は，すべて不稔であった (Fig. 4, Plate 2).

イソニガナにタカネニガナを交配することはできなかった．

b. タニガワニガナ×イソニガナ

　イソニガナ×タニガワニガナ

この両者の組合せでは正逆交配で比較的容易に雑種種子が得られる．雑種植物の形態は両親のほぼ中

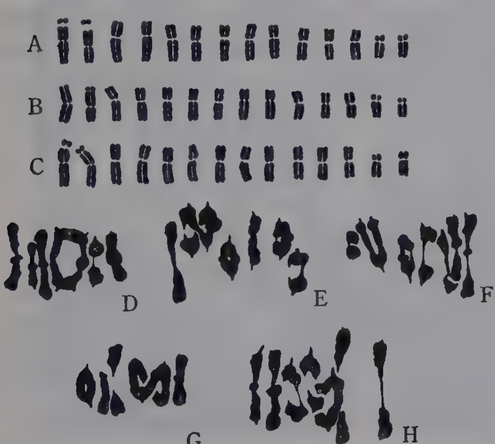


Fig. 4. Karyotypes and meiotic figures from F_1-hybrid of *I. dentata* subsp. *alpicola* × subsp. *nipponica*. ca. ×1000

A and B: Aberrant karyotypes, found in two different plants respectively. C: Unchanged somatic chromosomes, found in the other individual. A, B, C: $2n = 14$. D, E, F and G: Meiotic figures (at first anaphase) in the plant having the karyotypes A and B, respectively. H: Normal meiotic figure in the individual having the karyotype C.

D, E: 1Ⅳ+5Ⅱ, F: 1Ⅲ+5Ⅱ+1Ⅰ, G: 1Ⅵ+4Ⅱ, H: 7Ⅱ (Normal pairing).

間で葉は厚くなるが茎葉はイソニガナのようには丸くならない．体細胞染色体は 14 本で染色体には全く異常は認められず，花粉の稔性も 80% 以上と極めて高く，多数の種子が得られた．同株ではあまり結実率はよくない．戻し交配も行なわれ，タニガワニガナ，イソニガナいずれからも稔性の種子が得られた (Plate 1).

このように容易に交配が可能なことからタニガワニガナとイソニガナとの間には何ら生理的隔離はないと思われる．これに対してタカネニガナはイソニガナとの間に何らかの不均衡があるように思える．

考　察

2倍体，3倍体，4倍体のあるニガナ類で通常の両性生殖をする2倍体が高山と裏日本海岸という特別な環境に限られている事実と理論的に3倍体合成の要因となるべき4倍体が高山に単為生殖植物として残されている事実は何を物語っているのだろうか．竹本[6]) は低地に生育する海岸植物のイソニガナがニガナ類の原始型に近いかも知れないとのべているが，高山の2倍体や4倍体が日本が寒冷な頃のいわゆる“遺存種”で，これらから低地の2倍体3倍体が派生したのだと考えることもできそうである．しかし，ニガナ類の分化の過程には地質学的年代や日本以外の広大な大陸が条件として入っているだろうし問題はいつまでたっても推論の域に留まるのかも知れない．しかし現在高山と海岸に2倍体が残されている事実を発展させるためこの両者間の交配を試みることはニガナ類の分化を解明するささやかな手がかりともなろう．

高山の2倍体にはいままで知られたタカネニガナ以外にもう一つの植物があることが今回わかったのである．いわゆるタニガワニガナであって，この植物はニガナ類の分化を考える上で重要な位置を占めると思われる．高山植物と海岸植物との交配の結果，程度の差こそあれ完全な生理的隔離はみられないことが知られた．タニガワニガナとイソニガナは極めて近い関係にあるけれども，タカネニガナとイソニガナとの場合には少し事情が違うように思える．交配によって F_1 植物を得ることはできるが，不稔性であり，ときにはその細胞には染色体の動揺が観察されたのである．その動揺は仁染色体の形態変化，つまり仁染色体と染色体組を構成する他の染色体との

間の相互転座と二次狭窄の消失とであった．両親植物およびその子孫には実験の前後を通じて何等の変化もないのだから，雑種形成のさい何か細胞的不均衡が生じていたと解釈することもできよう．このような現象は自然集団中の3倍体植物でも観察される．しかしすべての雑種個体にもこのような染色体異常が見られたわけではなく，条件さえよければこのタカネニガナとイソニガナとの間には稔性の雑種が形成される可能性もあり得ると思われる．

ニガナ類を構成している植物のなかで一番強勢に分布している単為生殖性の3倍体が如何に形成されたかが興味の中心となる．自然にある3倍体と2倍体と形質を比較してみよう．全般的な外部形態，茎中部の葉，頭状花の数などから海岸性の2倍体イソニガナは3倍体のハナニガナと類似な点が多い．今回の実験で2倍体のタニガワニガナとイソニガナとの間には何らの生理的隔離がみられないばかりか，外部形態も似ていることが知られた．つまりハナニガナ，イソニガナ，タニガワニガナは同じ系列に属するものと考えられる．3倍体のニガナは，頭状花の小花数が6前後と上記のものより少なく，茎中部の葉の抱茎の程度も少ないようであり，少し違った印象をあたえる．高山性の2倍体タカネニガナは，外部形態上からも，生理的にもかなり違っているようである．これと類似の形態を示す3倍体植物を探すと，ずっと大型ではあるが，ハイニガナ (*I. dentata* var. *stolonifera* $2n=21$，西岡未発表) であろう．また紀伊の北山川の岩上でみつけられたドロニガナ (*I. dentata* subsp. *kitayamensis*) の外部形態はタカネニガナによく似ている．この植物は2倍体である (西岡 未発表)．分類上果実の形態が重要な形質として取扱われているが，タカネニガナとドロニガナはいままで列挙してきたニガナ類植物のなかでも特異な存在である．つまりタカネニガナにはそう果に長い嘴があり，またドロニガナのには嘴がほとんど認められない．外部形態上の特徴や果実の形態がニガナ類の分化の問題を解明するのにどの位手がかりになるか不明であるが，しかし検討されなくてはならない．

起源の不明な4倍体のクモマニガナや，新たに見出された3倍体の高山植物についても検討が加えられている．また人工4倍体や3倍体を合成することによって問題をより具体的なものにしようと努力している．これらの研究は続けられており結果は続いて報告されるはずである．

終りにあたり，終始御指導を頂いている小野記彦教授，ならびに分類上の御指導をいただいている京都大学理学部北村四郎教授，牧野標本館の水島正美助教授に深く感謝いたします．

文　　献

1) Kitamura, S., Memoirs of the College of Science, Univ. Kyoto. (1956). 2) Babcock, E. B., Stebbins, Jr., G.L., and Jenkins, J. A., Cytologia Fujii Jub, 188 (1937). 3) 岡部作一, Bot. Mag. Tokyo **46**: 518 (1932). 4) 竹本貞一郎，染色体 **21**: 747 (1954). 5) 竹本貞一郎，植物学会大会講演要旨: 10 (1954). 6) 竹本貞一郎，植雑 **69**: 325 (1956). 7) 西岡泰三，植雑 **69**: 586 (1956).

Summary

Some miscellaneous observations in the phylogenetic study of the *Ixeris dentata* group are preliminarily reported.

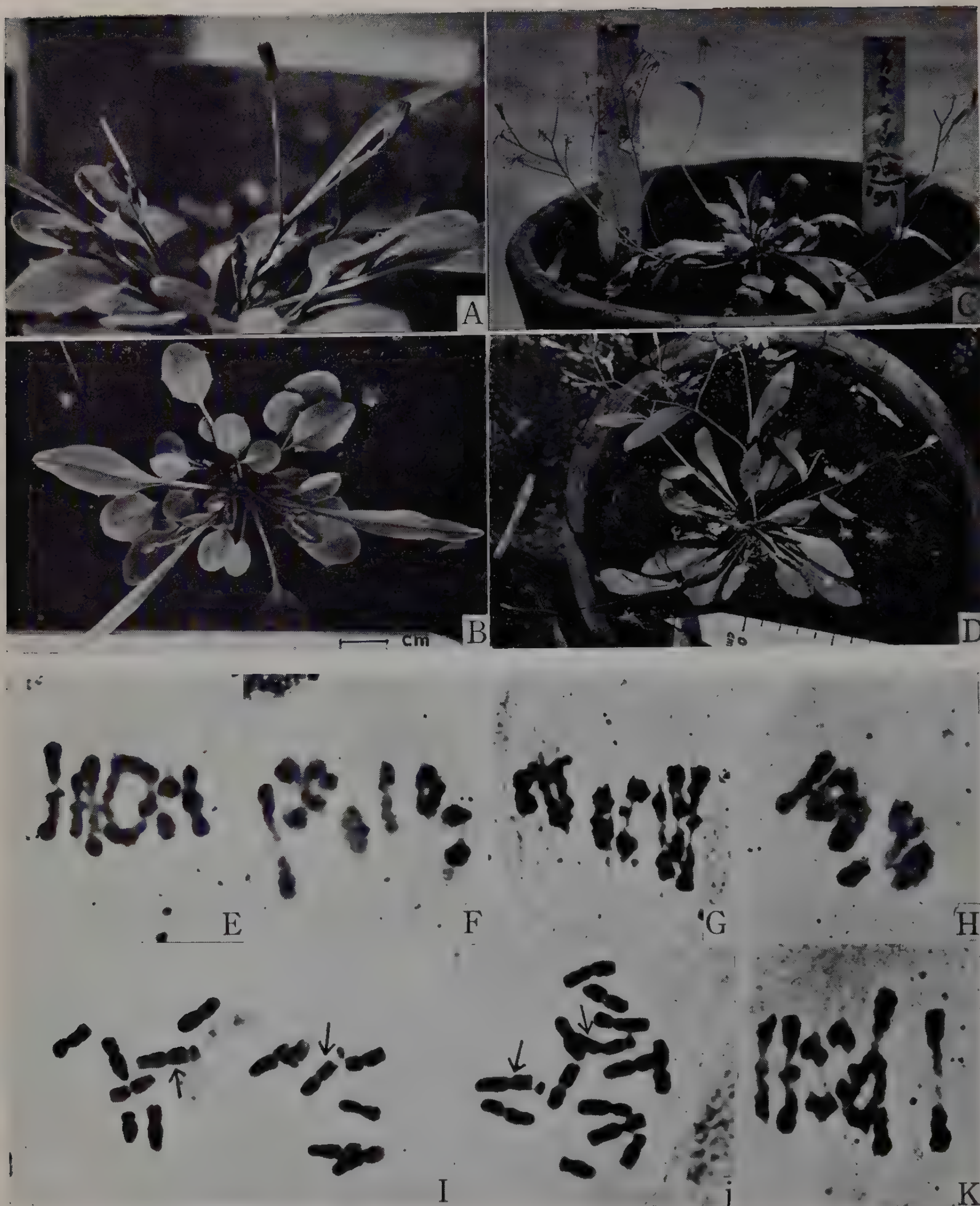
The *Ixeris dentata* group implies *I. dentata* and its subspecies or varieties, and the members of this group have the basic chromosome number seven. It has been reported that the sexual diploid plants are only found at the alpine and the seashore regions, the apomictic tetraploids are alpine in habitat, while the apomictic triploids are widely distributed from low lands up to low mountains in Japan.

1) *I. dentata* var. *albiflora* f. *amplifolia*, one of the triploid members, has different karyotypes according to different localities. This seems to apply also to the other triploid members.

2) A new type of diploid alpine plant was first found at Mt. Tanigawa. This plant is

A, B: *I. dentata* subsp. *nipponica*. C, D: "Tanigawa-nigana" × *I. dentata* subsp. *nipponica*. E. F: "Tanigawa-nigana".
A, C, E····Side view; B, D, F····Top view.

Plate 2.



A, B: *I. dentata* subsp. *alpicola*. C, D: *I. dentata* subsp. *alpicola* × subsp. *nipponica*.

A, C····Side view; B, D····Top view.

Meiotic and mitotic chromosomes of the above mentioned hybrid (cf. Fig. 4).

E, F, G, H, I: Chromosomes from the individual having aberrant karyotype. J, K: Chromosomes from the individual having normal karyotype.

Arrow indicates nucleolar chromosome. ca. ×1600

temporarily named "Tanigawa-nigana" in this paper.

A triploid plant which resembles the usual tetraploid alpine plant, was found in the South Alps of Japan.

3) The crossing between the alpine plant and the seashore one which are geographically isolated each other, was undertaken.

As the alpine parent, *I. dentata* subsp. *alpicola* and the above mentioned "Tanigawa-nigana", and as the seashore parent, *I. dentata* subsp. *nipponica* were used. The F_1-hybrid of "Tanigawa-nigana" × *I. dentata* subsp. *nipponica* was easily obtained. It was fertile and its external morphology showed the intermediate characters between those of parents. But F_1-hydrid of *I. dentata* subsp. *alpicola* × subsp. *nipponica* was hardly produced, and when obtained, all of the F_1-individuals were sterile and their external morphology was rather similar to that of the alpine parent. In two of five survivals of the latter hybrids, some chromosome aberrations occurred.

Bot. Mag. Tokyo, **73**: 438 (October 25, 1960)

Short Communication

TETSUO KOYAMA*: Some Transfers of Names Related to Cyperaceae

小 山 鐵 夫: ヒンジガヤツリ属について

Received August 9, 1960

Lipocarpha, a small cyperaceous genus, has hitherto been placed near *Hypolytrum* of the subfamily Mapanioideae, but my morphological investigations led me to the conclusion that *Lipocarpha* is a special group of *Cyperus* systematically closest to *Kyllinga*. In my opinion, of the two so-called inner scales, the adaxial one is a prophyll, whereas the abaxial (or the upper) one is a floral scale. Thus, floral unit of *Lipocarpha* is a dorsiventrally compressed *Cyperus*-type spikelet. The full interpretation and discussion are given in a separate monograph of Asiatic Cyperaceae together with technical transfers of related names. I have picked up here some of them for the immediate needs.

1. **Cyperus ceylanicus** T. Koyama, nomen novum.—*Hypaelytrum sphacelatum* Vahl, Enum. Pl. **2**: 283 (1806), non *Cyperum sphacelatus* Rottb. (1773)—*Scirpus hemisphaericus* Roth (1821), non *Cyperus hemisphaericus* Böcklr. (1859)—*Hypolytrum ceylanicum* Heyne ex Nees in Linnaea **9**: 288 (1834), nomen nudum—*Lipocarpha sphacelata* (Vahl) Kunth (1837).

2. **Cyperus echinolepis** T. Koyama, nomen novum.—*Lipocarpha albiceps* Ridley in Trans. Linn. Soc. Ser. 2, **2**: 163 (1884), non *Cyperus albiceps* Ridley (1884).

3. **Cyperus fimbristyloides** T. Koyama, nomen novum.—*Lipocarpha paradoxa* Chermezon in Bull, Soc. Bot. France **68**: 425 (1921)—*Mariscus paradoxa* (Chermez.) Chermezon (1925).

4. **Cyperus Lipocarpha** T. Koyama, nomen novum.—*Scirpus chinensis* Osbeck, Dagb. Ostindisk Resa, 220 (1757), non Munro (1857)—*Lipocarpha argentea* (Vahl) Kunth (1837)—*Lipocarpha senegalensis* (Lamk.) Th. & Hél. Durand (1909), non *Cyperus senegaiensis* Mattf. & Kükenth (1937)—*Lipocarpha chinensis* (Osbeck) Kern (1958), non *Cyperus sinensis* Debeaux (1877).

5. **Cyperus persquarrosus** T. Koyama, nomen novum.—*Lipocarpha pulcherrima* Ridley in Trans. Linn. Soc. Ser. 2, **2**: 162 (1884), non *Cyperus pulcherrimus* Willd. ex Kunth (1837)—*Lip. atropurpurea* Böcklr. (1888), non *Cyperus atropurpureus* Pers. (1805)—*Lip. tenera* Böcklr. (1888), non *Cyperus tener* Vahl (1806).

6. **Cyperus neo-Barteri** T. Koyama, nomen novum.—*Lipocarpha Barteri* C. B. Clarke in Dur. & Schinz, Consp. Fl. Afr. **5**: 650 (1895), non *Cyperus Barteri* Böcklr. (1868)—*Kyllinga baoulensis* Cheval., Expl. Bot. Afr. Occid. Franc. **1**: 698 (1920), non *Cyperus baoulensis* Kükenth. (1931).

7. **Cyperus Prieuriana** (Steud.) T. Koyama, comb. nova.—*Lipocarpha Prieuriana* Steudel, Synops. Pl. Gl. **2**: 130 (1855).

8. **Cyperus Sellowianus** (Kunth) T. Koyama, comb. nova.—*Lipocarpha Sellowiana* Kunth, Enum. Pl. **2**: 267 (1837).

9. **Cyperus submaculatus** T. Koyama, nomen novum.—*Hypolytrum argentea* H. B. K., Nov. Gen. & Sp. Pl. 218 (1815), non *Cyperus argenteus* Ridl. (1884)—*Kyllinga maculata* Michx., Fl. Bor.-Amer. **1**: 29 (1803), non *Cyperus maculatus* Böcklr. (1864)—*Lipocarpha Humboldtiana* Nees (1834), non *Cyperus Humboldtianus* Schult. (1824)—*Lip. maculata* (Michx.) Torrey.

10. **Cyperus unistamen** T. Koyama, nomen novum.—*Lipocarpha minima* Chermezon in Bull. Soc. Bot. France **68**: 425 (1921), non *Cyperus minimus* Linn. (1753).

11. **Cyperus Zollingeriana** (Böcklr.) T. Koyama, comb. nova.—*Lipocarpha Zollingeriana* Böcklr. in Flora **42**: 100 (1859)—*Lipocarpha microcephala* (R. Br.) Kunth (1837), non *Cyperus microcephalus* R. Br. (1810)—*Isolepis squarrosa* Miq. (1865), non *Cyperus squarrosus* Linn. (1756)—*Ascolepis kyllingioides* Steudel (1855), non *Cyperus kyllingaeoides* Vahl (1806).

---

* Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan

Bot. Mag. Tokyo **73**: 439–444 (October 25, 1960)

雑　　録

植被の物質生産に関する国際生態学シンポジウム短報

宮　脇　昭*

Ein Bericht von dem Internationalen Ökologischen Symposion über die Stoffproduktion der Pflanzendecke in Stuttgart-Hohenheim, 4–7. Mai 1960.

von Akira MIYAWAKI*

Hohenheim bei Stuttgart で本年5月4日から7日にわたり Prof. Dr. H. Walter, Dr. H. Lieth の主催により，物質生産に関するはじめての国際シンポジウムが開かれた．10か国約40人の研究者が植物生態，社会学，農林学の各分野から参加して，14講演（別に夜は幻燈を主とした一般講演3）を中心として討議された．

各研究者の専攻分野によって，物質生産に関する研究の目的・方法・見方がまだかなり異なっている印象を受けたが，それなりに今後のこの分野の研究発展に共通の基盤を見出す端緒を開いたものとして意義深いものと思われる．以下簡単にこのシンポジウムについて報告したい．わが国の関連分野の研究者への幾分の刺戟となれば幸いである．

4日間のシンポジウム期間中実際に行なわれたのは第2・3日であった．Prof. Dr. Th. Schmucker, Hann.-Münden (Deutschland), Prof. Dr. D. Müller, Kopenhagen (Dänemark), Prof. Dr. J. H. Becking, Wageningen (Holland), Prof. Dr. L. Fenaroli, Bergamo (Italien), Prof. Dr. E. Aichinger, Klagenfurt (Österreich), Prof. Dr. H. Walter, Hohenheim (Deutschland) により，I. Allge. Produktionskalkül und forstliche Probleme, II. Probleme bei speziellen Vegetationseinheiten, III. Grünlandkolloquium の3分科に分けて講演討議された．

第1夜は前夜祭の意味で，夜の会食後2講演が行なわれた．すなわち18時会食の後，Walter から内外国からの参加者に対する歓迎の挨拶がユーモアを交えて行なわれた．

その後 D. Müller, Kopenhagen から Boysen-Jensen und die Stoffproduktion der Pflanzen と題して，1959年11月21日死去した彼の前任者 Prof. Dr. Boysen-Jensen の功績と研究態度を讃えた．Boysen-Jensen は偉大な理論家であり実験者であった．現代のデンマルク植物生理学の基礎をきずいたのは彼であった．彼は物質生産の問題を高等植物に試みた最初の人である．1910年はじめてデンマルク語で物質生産に関する論文を書いて以来，死去の年まで85の論文を書いている．彼は76年のその生涯を主として Lichtfaktor と Stoffproduktion の関係究明に捧げた．さらに Stoffproduktion と Nettoproduktion の関係について深く研究している．Photosynthese 解明法も彼の手により次第に改良されてきた．——彼のこの分野の研究をまとめた最初の Wuchsstoffe についての Monographie "Die Wuchsstofftheorie und ihre Bedeutung für die Analyse des Wachstums und der Wachstumsbewegungen der Pflanzen, G. Fischer, Jena 1935" はわが国でも広く読まれている——筆者註．さらに死の年まで彼は，植物生育期間中の Determinierung と Differenzierung，細胞の生と死の間の相違などについても研究した．Boysen-Jensen 自身主著と称した Det Levende (Das Lebende) Bd. 1–4. 1951–'53 に彼は記している．"無機物的自然の中においてはすべての変化は，お互いに何の Zusammenhang も Plan もなく，ただ一定の Kausalprinzip のもとに進行する，しかし，すべての生物

---

* 横浜国立大学生物学教室　Biologisches Institut, Staatliche Universität Yokohama, Kamakura, z. Zt. Bundesanstalt für Vegetationskartierung, Stolzenau/Weser, Deutschland.

は，それと対象的に1つの Ganzheit として，生物内のすべての Lebensäußerungen の生因と保持の間における一つの harmonisches Zusammenspiel を通して形成される” と記している．この „Ganzheitsbetrachtung” こそ，この本の Ursprung である．晩年の彼は自身の体験を通じて以下のことを，とくに深く認識した．いかに分析を深く Zellprozesse の中に進めるか，“aber vergiss über die Analyse nicht die Synthese, über Einzelheiten nicht die Ganzheit, denn erinnere: Die Pflanzen sind lebend”.

この Müller の講演は，すぐれた Boysen-Jensen の生物学究明の基礎的考え方とともに参会者一同の深い共感を呼んだ．

その後 W. Lötschert, Hamburg の幻燈による Die Vegetationsverhältnisse in Westcuba と題しての講演があった．彼は美しいスライドを基礎に半年間のキューバ島西部の植生調査結果を，同地環境条件資料と比較概観した．

第2日，I．一般的生産計算と林業的問題，

まず Hohenheim 農業大学学長 Pflugfelder 教授の短かい挨拶の後，正式のシンポジウムが開かれた．

S. S. Paterson, Lerum (Schweden) は，Der CVP-Index als Ausdruck für forstliche Produktions-Potentiale と題して，気候条件から世界の森林生産潜在能力を決める試案を述べた．すなわち，Wärme・Feuchtigkeit・Länge der Vegetationszeit が Photosyntheseleistung を決定するものとして，CVP-Klimabedingte Vegetationsform-Produktivität 式を提案した．彼は各地の気象台による数値より CVP-Indexwerten (Klimatisophyten) を計算，世界地図の上に等価点を線で結んだ．同じ計算値（指数）地域を気候的見地から見た等しい生産能力を持っている地域とし，Phyochoren と称した．この CVP-Index により気候と潜在森林生産能力の間には密接な相関関係があると述べた．

この講演には同夜の総合討論で各方面の研究者から，かなり批判的質疑があった．とくに Becking, Volk, Walter らから熱帯地方の森林生産構造は，暖・温帯のそれとはまったく異なる．スエーデンでは適するかも知れないが，その公式を全世界に応用しようとするのは無理である．数式も完全ではないと発言した．さらに Schlenker から気候条件を抽象化するのは一般に非常に困難である．とくにドイツのごとく同じ気候条件下でも，土壌が各地によってまったく異なるところで，気候条件のみで森林生産能力を推定しようとするのは不合理である．Knapp らより，おのおのの種によって温度への反応はまったく異なる．植物各種について，個々の適応性の研究なく，いきなり大ざっぱな月平均の気温ですべての森林生産力を把握しようとするのは研究が飛躍しすぎている．最後に Aichinger からこの講演の題目は，「林業的気候空間を決定する手段としての CVP-指数」とした方がよいと発言した．

D. Müller, Kopenhagen, Wie groß ist der prozentuale Anteil der Nettoproduktion an der Bruttoproduktion.

彼の講演の本質は，1954 年の彼等の論文*が骨子となっていた．

デンマルクにおけるブナ林 (*Fagus silvatica*-Wälder) における全生産量 Bruttoproduktion と，それから呼吸・落葉などによる損失を差し引いた絶対的生産量 Nettoproduktion の年間における ha 当りの乾量物質との関係を計算・図示して，その量的関係を説明した．Bruttoproduktion は ha 当り年間 23.5t に達し，40〜60 年生のブナで最高に達する．葉の Nettoproduktion は Bruttoproduktion－(葉の損失＋葉の呼吸による乾量物質の損失）である．葉の Nettoproduktion は，ha 当り 16.2t に達し，その最高生産量は同様に 40〜60 年生樹木に期待される．全 Bruttoproduktion から根・幹・葉の呼吸による乾量損失および根・梢・葉の損失は 60%におよぶことが計算により明らかである．残余の Bruttoproduktion が根・幹・梢の生長にあてられる．

地球の約 10% は熱帯林に属し，また，全森林の 38% は同じく熱帯林に属している．同じ研究基盤に立った熱帯林の研究はきわめて重要である．

G. Schlenker, Stuttgart, Ertragspotentiale verschiedener Waldgesellschaften Südwestdeutschlands.

造林計画は気候的・地質学的および立地の歴史的見地に立って行なわれなければならない．自然植生

* Möller, Müller u. Nielsen 1954: Ein Diagramm der Stoffproduktion im Buchenwald, Ber. Schweizer. Bot. Gesellsch. **64**: 487-494, Bern.

は地域的な気候に相応しているという観点に立って Regionalgesellschaft・Standortseinheit・平均総乾量生産物の関係を kontinental-montane ブナ林と eichenreicher Laubwald に分けて比較した. Fichte (*Picea excelsa* (Lamk.) Lk.) の生産量は一般にブナより高いが, Standortsgesellschaft がブナ林である立地では人為的保護なくしては, 生育がきわめて悪い. また Fichte の人工林を行なうと土壌が非常に酸性に傾き, 立地の将来の生産能力を減退させる.

夜の総合討論の際, Standortsgesellschaft とは何か, R. Tüxen はじめ多く植物社会学者のいう潜在自然植生 Potentielle natürliche Vegetation の Synonym と解称してよいか, どのような基盤に立って把握可能か, との筆者および Eskuche の質問に対して Schlenker はつぎのように答えた. Synonym と考えてもらいたい. 地図の作成にあたっては, まず floristisch に種の組合せにより画く. 植被のないところでは, 土壌の側からその形態的特性により画く.

W. Tranquillini, Innsbruck; Die Vergleich zwischen Produktion und Assimilation. ある植物の生育中の物質生産量を知るためには各種の方法が存在している. すなわち Dendrographen による樹幹の肥大生長記録法 (Fritts and Fritts 1955), 年間の各時季における任意抽出法による生長増加量の乾量による決定 (Gregory 1917, Rutter 1957), または放射線を側面照射し, その吸収の測定による Massenzunahme の調査 (Unger 1959) などが挙げられる.

個々の植物の物質生産量の厳密な分析は, その CO_2-Umsatz (同化と呼吸) の継続的測定により行なわれる. この方法により *Pinus cembra* の幼木について Tirol (オーストリア) の自然生育地で調査した (Tranquillini 1959).

すなわち 1954～1955 年にわたる1年間, 植物の地上部における昼間・夜間および冬期積雪下の呼吸により生じた CO_2 の全量を測定した. さらに実験室で, 各種気温下の根系の呼吸量についても測定した. 土壌湿度に対する知見により, 年間の根系呼吸の全量を計算した. 演者はさらにこれらの光合成・呼吸による植物体各部の CO_2 の収支を表により示し, 差し引年間の CO_2-Überschuss (剰余額) を 4837 mg CO_2-g Nadeln とした. 実験に使われた *Pinus cembra* では, 年間を通じて 1g の CO_2-Überschuss は 2.2g の乾量増加量となる. このガス交換測定値から計算された生長量は, 同じ年の直接測定された生長量よりもはるかに大きい. これは測定された植物と, この植物と共生している菌類 (Mykorrhiza) による同化量が含まれているからである.

K. H. Kreeb, Hohenheim; Hydratur und Ertrag bei Monokulturen. 生産量を決定するもっとも重要な要因の1つは水分である. しかも Walter 1931 年以後植物にとって重要なのは水分の量ではなく, 水分状態 Wasserzustand=Hydratur (für die Pflanze) が決定的要因となった. と演者は前おきして彼の研究結果を述べた. 彼は種々の耕作植物の滲透価を測定した. そしてこの滲透価を Hydratur (水度) として表現した. 植物の滲透価 (=Hydratur) は水分経済に関係している. 彼は農作物の Hydratur と土壌の水分保持力 Bodensaugkraft との関係, さらに農作物の収穫量について研究した. Bodensaugkraft の増加とともに細胞液の滲透価は上昇する. それに呼応して収穫量は減少する. この例として *Brassica*, *Hordeum* は他の農作物より高い土壌の塩分濃度に耐え得る. さらに演者は自然植生の生産量解明への手段としての Hydratur 利用の可能性についても言及した.

W. Haber, Münster; Über Zusammenhänge zwischen der Produktivität eines Pflanzenbestandes und der Bodenatmung. 土壌への CO_2 の供給と遊離の Bilanz (差引高) から植生の生産量を究明する1つの試ろみについて述べた. 土壌への CO_2 の供給はまず土中微生物の物質交代による. ついで根系の呼吸により行なわれる. したがって植生の物質生産量は, 土中の CO_2 の量ときわめて密接な関係にある. 演者は土壌の CO_2 遊離と植物の乾量生産量の関係を C (Kohlenstoff) の重量単位で表現した.

第3日. E. Aichinger, Klagenfurt; Wald- und Wiesenentwicklungstypen als Grundlage der Produktionsleistung. 演者はいう. "各種 Wiesen, Weiden, 森林の生産能力は pleogenetische 植生単位として, 把握しなければならない. すべての植物群落は, それぞれ固有の発展段階を経て現在に到っている". すなわち彼は多くの群落を群落遷移途上

の，ある段階 Stufe として把握している．

現在牧野となっているオーストリア東南部の各草原と本来の森林と比較して，各群落の遷移・退行の関係を模式化して示した．現在草原化している群落単位の把握には，かつて森林であった当時の種の組み合せとの比較からなされねばならぬ．森林の一齊乱伐や，牧野に肥料を施すことなく常に乾草を穫ることは，自然の Nahrstoffkreislauf をいちじるしく妨げ，植生の生産量を減退さす．

R. Knapp, Gießen, Experimentelle Untersuchungen über Faktoren der Ertragsbildung in Pflanzenbeständen. 群落の Ertragsbildung を決する主な要因としては，今までしばしば研究されてきた水分・光エネルギー・土壌養分のみでなく植物の特別の Reaktion と個々の植物が群落化するにあたって，お互いに受け合う相互作用がきわめて本質的な影響を与える．演者は *Artemisia* 類などの薬用植物， *Lolium*・*Phleum* などの Weide 植物，さらに *Hordeum* などの農作物類を材料として，実験的に同種間および種間の競争現象解明を試みている．

Reinbestand では，お互い個体間に被害を受けることなく，かえって生産量が増加する場合と，逆に減少する場合がある．これら同種内の個体間の＋・ー の関係を究明するため，各種の混植実験を行なった．混植の場合が単植の場合より生産量が増加する場合と減少する場合がある．この場合，種間の相互影響のみでなく，環境条件たとえば土中の窒素分が大きく影響する，と述べた．

Walter の問いに答え Knapp は，競争現象と多くの環境条件を同時に実験的に研究するのは，現段階では，まだ困難であると答えた．さらに彼は Lieth の最近オーストラリアの学者や日本の吉良らによってこの種研究が進められているが知っているか．の質問に対して，じゅうぶん参考にしていると述べた．

Rademacher は彼の助手とともに Über die gegenseitige Beeinflussung von Ackerunkraut und Kulturpflanzen, insbesondere *Sinapis arvensis* auf *Triticum vulgare* について簡単に紹介した．さらに休憩時間に水耕栽培によりポット試験中の彼の実験温室に希望者を案内した．

K. Walther, Stolzenau; Über die Eichung von Pflanzengesellschaften auf ihre Erträge. 最近数十年間の植生地図研究の発達により，現実植生 Reale Vegetation および潜在自然植生 Potentielle natürliche Vegetation を広地域にわたって植生地図に描くことが可能になった．もしすべての，または重要な群落に対して Signifikante Ertragswerte が見出されるならば，その地域の植生生産量について概観を得ることができる．

森林や牧野のように人間がその植物の大部分を利用する群落では，Ertragswert は植生地図上の各群落単位から問題なく把握できる．問題は耕地の雑草群落にある．耕地では永続的な各種の管理による極度の人為的影響が，自然環境条件の上に覆いかぶさっている．このような立地の雑草群落の植生単位から，雑草と競争関係にある農作物の収穫量を推定することができるだろうか．

演者はこの問題を解決するにあたり，隣接している管理状態はまったく同じであるが，雑草群落の異なる２つの区画を各地に選び，お互いの収穫量を調査した．この場合の両群落地からの収穫量の相違には，もはや特別人為的要因は考える必要がなくなる．自然環境条件と（雑草）群落と結びついた要因のみが反映される．この結果から群落を Eichung として生産量を調べる場合，生態学的条件とくにドイツでは地下水の高さが耕地雑草の形成に大きな影響を持っていることがわかる．測定された (geeichte) 群落の生産量と土中水分の Stufe を包含した植生地図から “Wasserstufenkarte” が導かれる．この地図から植生の収穫能力とその立地の土壌温度との関係が明らかとなり，農業や Wasserwirtschaft 上の問題解決に役立つであろう．

II．特殊な植生単位についての問題

F. Gessner, München; Die Primärproduktion der Hydrophytengesellschaften (Plankton und Benthos) in Binnengewässern und im Meere. 最近発刊された彼の Lehrbuch, Hydrobotanik 第２巻 701 pp. 1959 の主な点の紹介というべき講演であった．まず水生植物群落は種の組合せ Artenverbindung によって把握することができる．Phytoplankton の群落構成の解明は，高等植物群落に比して容易である，と前おきして，淡・海水生微小植物群落を植物社会学的・生態学的な面から世界中の文献を基礎に説明した．宝月・市村など日本の研

究者による業蹟も紹介・批判された.

L.C. Bliss, Urbana (USA); Net Primary Production of Tundra Ecosystems. Tundra 植物の単位面積当りの生産能力に関する研究は比較的少ない. 演者はまず極地地方および高山帯の Tundra 植生生産能力について各研究者による今までの研究結果を比較紹介した. 極北地方の Tundra 植生の単位面積当り生産量が, 高山帯のそれに比して一般に高いのは, 光合成作用の行なわれる夏季の日照時間が長いこと. 環境条件も前者が後者より恵まれていると述べた. その後 Mt. Washington の Tundra 植生を演者自身の資料に基づいて述べた. 夏季, 植物生育期間中の継続刈取り調査 (刈取り区を常に変える) による生産量をカーブで示すと2つの高い山が見られる. 第1の頂点は, 初夏の植物群落の最初の活動期に見られる. 第2の頂点は晩夏に見られる. 盛夏の生産能力は一般に低い. この原因として第1の, 植物活動期の生産量の増加は根部に貯えられていた栄養分による. 盛夏の生産量低下期間は開花および初期の結実期と一致する. 晩夏の生産量増加の原因として, この時期は果実はすでに結実し, 植物は好都合な気温と土壌水分条件下に最高の光合成作用が行なえるためであると述べた (この問題については講演後, Bornkamm, Lieth, Walter らからかなり批判された). また Tundra 地帯の植生の生産量が, 暖・温帯のそれに比して低いのは, 生育期間が短かいことが主な原因であるとした.

III. 緑野についての Kolloquium

D.M. de Vries, Wageningen (Holland); Trockenmassenertrag und Bewertung von Dominanzgesellschaften. 演者はいう. 牧野利用上の見地から重量の多い優占種gewichtsmäßig vorherrschenden Arten は, 植物社会学上の観点から重要であるごとく, 農学上もきわめて重要な意味を持っている. 各 1/4 dm² の刈取り乾量の比較によって, 緑野群落をある種の優占度が絶対的であるか, あるいはどれだけの割合を占めているかによって4区分した. すなわち sehr rein, rein, unrein, gemischt. 主な種の優占群落から, その立地の生態学的条件がある程度推定される他に, 緑野の農・牧畜への利用価値判断の有力な資料となる. オランダの緑野における利用価値の高い優占種は, *Lolium perenne*, *Poa trivialis*, *Poa pratensis*, *Alopecurus pratensis* および *Festuca pratensis* である. この中 *Poa trivialis* の優占群落は, 乾量の年間収穫量がもっとも高い.

W. Krause, Donaueschingen; Über des Produktivitätspotential der Allmendweiden im Schwarzwald. 彼の講演はわが国でも重要な課題の一つである山岳牧野の改良について興味ある研究成果を述べた. 南ドイツ, Schwarzwald の山岳・亜高山地帯の *Nardo-Callunetea*-Weiden は高い生産能力 Leistungspotential を持っているにもかかわらず, 現在協同牧野になっている関係もわざわいして, 極度の粗放経営のもとにきわめて僅かな産業的収穫量 Wirtschaftsertrag しか得られない. 8か年におよぶ窒素・燐酸・カリの施肥試験の平均結果によると, ヘクタール当りの乾草収穫量は, 現状のままでは 1500 kg のものが 5500 kg 期待される. この現象が試験地附近の局地のものか, Schwarzwald 全体についていえるものかを, 植生地図の比較により調査した. さらに各立地の生態条件をも加えて吟味した. その結果 *Nardus stricta* L. の豊富に出現する, 地形的には凹面または平面に高い生産能力 Ertragsleistungen を持っていることがわかった. この立地は褐色土壌構造を有し, 土層が厚く, 根群が深く発達することができる. さらに春季まで積雪が長く続き, 冬季に極端な気候変動が少なく, 夏季湿潤である.

この講演に関連して Aichinger から略奪農法の盛んな今日, 牧野にも施肥, とくに有機質肥料のたえざる供給はきわめて重要である. 冬季の積雪は植生への極端な寒気を避けるため, とくに牧野に対しては重要な意味を持っている. われわれは積雪を長く保たす方法について考えねばならぬと強調した.

Th. A. de Boer, Wageningen (Holland); Produktion und Produktionsverteilung von Grünlandvegetationseinheiten. われわれの植生区分 Vegetationseinteilung の基礎は種の組み合せとその量的配分にある. これはさらに土壌の水分・pH・管理状態など各種の草原の生育条件の Komplexe への指標価値 Zeigerwert を持っている. オランダの大部分 *Lolieto-Cynosuretum* および一部 *Molinion coeruleae*, *Bromion racemosi* に属している草原について, その下級植生区分単位と収穫量について比較調査を行なった. その際2つの調査法を併

用した. 第1の方法は，生育期間中に5週間おきに5回刈取り調査を行ない (Rohertrag)，その資料から1日当りの乾量生産量を算出した. 第2の方法はGeith の Normen から換算した (Reinertrag). これらの研究から種の組成により下位区分まで分けられた植生単位から明らかに，各群落単位の平均純収量と標準総収穫量を知ることができる.

第3日夕は 20.00 時から H. Walter によるカラースライドを主としたオーストラリア大陸の植生について通俗講演が行なわれた.

第4日は，午前11時まで綜合討論が行なわれた.

今までの講演について，主として研究上の技術的問題が,各研究者の専門分野の立場から行なわれた.

筆者は物質生産・競争を生態学的立場から主として研究している門司・宝月・吉良らの日本の現在の代表的研究者および研究室について簡単に紹介した. 座長の Walter は今まで日本の研究者の成果は，ヨーロッパでは一般にじゅうぶん知られていなかったので，とくに筆者の紹介に感謝する旨発言した.

最後に Walter から各研究者の専門分野によって生産量に対する見方が異なる. 林学者は木材生産量を，牧野では草生量を，耕地では農作物と人間の利用し得る部分のみを生産量と考えている. しかし理想的には Bestand の gesamte Trockensubstanz を，その生産量と考えるべきである. 応用植物学の研究者達も全乾燥量を生産量と考えるようにすることはきわめて重要である. それによって理論と応用の両分野における生理・生態・社会の各分野の研究者が同じ基盤に立って研究を進める道が開けるであろうと結んだ.

その後全員バスにより Uracher Wasserfall への Exkursion に参加，なごやかな半日を過した.

植物生態学・社会学の両分野を通じて理論的にも応用的見地からもきわめて重要な課題の一つである物質生産についての，この国際シンポジウムを通じて筆者に感じられたことは，各分野の研究者がようやくこの問題に真剣にとり組み始めたことである. 各講演者はいずれもお互いの専門研究分野内での物質生産問題について考え，まだ統一した共通基盤を持つに至っていない印象を受けた. しかしこれは，おそらく始めてのこの種シンポジウムのためで，時とともに個々の分析的研究結果が植物群落解明への Synthese に導かれることであろう.

全講演を通じていえることは，必ずしもすべての物質生産の研究が，厳密に定義づけられた植物群落をその Grundlage としないで，研究対象が漠然としているものと見受けられた. 植生の物質生産の研究は，今後他の多くの群落生態学的研究課題と同様に，厳密に定義づけられた植生単位を基礎として進められた時，その成果が各分野の研究者に容易に理解され，応用分野への貢献も容易に行なわれ得るのではなかろうか.

なおこのシンポジウムの講演および討論要旨の特別出版の見通しは，現在不明である.

Zusammenfassung

Vom 4.–7. Mai 1960 fand in Stuttgart-Hohenheim (Deutschland) ein Internationales Ökologisches Symposion über die Stoffproduktion der Pflanzendecke statt.

Mehr als 40 Fachleute der Pflanzenökologie, Pflanzensoziologie, Landwirtschaft und Forstwirtschaft kamen aus 10 verschiedenen Ländern hier zusammen.

Während der Tagung wurden 14 Referate aus den verschiedenen Fachgebieten, sowie an 2 Abenden 3 öffentliche Vorträge gehalten.

Wenn auch jeder nur aus den Kenntnissen seines engen Spezialgebietes urteilen konnte, kamen bei diesem ersten Versuch, das Problem der großräumlichen Stoffproduktion der Pflanzendecke näher zu erfassen, doch wichtige Ergebnisse und so viele Anregungen zusammen, daß sich für die Zukunft daraus eine ganzheitlichere Betrachtungsweise ergeben könnte, die für eine wirkliche Zusammenarbeit an diesem umfassenden Komplex von größtem Nutzen wäre.

Störend machten sich besonders neben dem Mangel an zuverlässigem und exaktem Zahlenmaterial die verschiedenartigen Bezugsgrundlagen bemerkbar. Verfasser schlägt darum vor, bei künftigen Untersuchungen eindeutig definierte Pflanzengesellschaften zugrunde zu legen, die einen genauen und wiederholbaren Vergleich gewährleisten.

抄　　録

植物形態形成における方法

Wardlaw, C. W.: Methods in plant morphogenesis. Jour. Linn. Soc. Lond. **56**: 154～169 (1959).

Morphogenesis には多くの研究方法と観察方法が考えられるが，最終目的は接合体からの分化を充分説明するにあるであろう．1) われわれは植物の形態について莫大な知識を有するにもかかわらず，さらに多くの研究が強調される．それはこの分野での正確な予備知識なしにはこれと関連のある生理学的問題の公式化や，適切な実験的あるいは分析的プログラムは考え出され得ないからである．2) 形態形成には一般にいちじるしい規則性があり，その要因を観察する有用な方法として手術が考えられる．しかしここで形を変えた多くの方法を制限する要がある．一方この規則性に着目し，Schüepp (1952), Abbé & Stein (1954) および Richards (1948, 1951) らは生長ならびに形態形成の研究は数学的解析方法によって如何に光明を与えられるものであるかを示した．3) すべての形態形成は物質代謝を伴なうものであり，それと関連する生化学，生理学の知識が頂端生長とか，形態形成のより正確な理解の基礎となるのは明らかである．しかし，現段階ではそのうち有用なのはごく僅かで培地の改良に注意がはらわれたに過ぎない．(a) 組織培養についての多くの研究のうち，形態形成の過程を直接に解明しているのは極めて少ないが，ある物質を標準培地へ附加すると根や芽の形成が誘導されるという発見は極めて重要であった．(Skoog, 1954; Skoog & Miller, 1957; Skoog & Tsui, 1948). (b) 頂端に直接あるいは間接に行なった化学処理で影響される初期の変化をさらに詳しく観察する必要があり，培地に加えた特殊物質が変化を期待する部分に到達しているかについて注意すべきである．4) 今日の生理遺伝学，形態形成の研究者は embryonic region で，何時，如何に特殊な遺伝子が行動するか，初期の形態形成の型ならびにそれに続く伸長が展開されるかという疑問を持っている．これは明らかに困難な問題で，まず胚の形態の詳細な比較観察から始めるべきである．材料は遺伝学的に研究されたもの，また物質代謝に特徴的差異のあること，あるいは生長が特殊な遺伝子に関係していることが望まれる．放射性物質，突然変異誘起剤の影響の研究はこの分野での最先端を行くものの一つである．5) 種の間には多かれ少なかれ形態の差異がみられる一方，全然類縁のないものの間で相同ということがある．これは単に進化学の大きな興味ある事実であるのみでなく，形態の起因考察における最も重要な基本的なものである．　(島袋敬一)

イワヒバの葉緑体の電子顕微鏡的研究

Kaja, H.: Elektronemikroskopische Untersuchungen an den Chloroplasten von *Seraginella Martensii* Spring. Ber. Dtsch. Bot. Ges. **72**: 311～320 (1959).

従来知見の少なかったシダ類葉緑体の微細構造がイワヒバの一種類について興味深い報告がなされている．20×2.5～5.1μ 程の盆状の葉緑体は光に向いた上側では大きな澱粉粒を下側では小さな多数の澱粉粒を含み，30～50 Å の二重膜および中間層からなる厚さ 60～150 Å の葉緑体膜によってとり囲まれ，その下には構造のよく分らない Peristromium が周辺を縁どっていて下側では 100～150 Å 程の厚さにすぎないが光に向いた上側では 400～800 Å で常に明瞭に巾広く葉緑体膜に平行に走っている．Haberlandt (1905) が上側では Plasmahaut があるが下側では見わけられないといっていたのは明らかにこの Peristromium を見ていたのである．葉緑体の内部は 80～100 位のラメラが密に並んで走り，各ラメラは 30～80 Å 程で相互の距離は 50～70 Å である．葉緑体の上側では Peristromium のすぐ下に殆んど葉緑体膜に平行に走っているが下側ではこの方向が次第にずれて最下部では多かれ少なかれ斜に葉緑体膜に鋭角をなして接近し Peristromium のところでその末端が終っている．葉緑体膜から遠ざかるとラメラは葉緑体全体を貫通しているのではなく内部ではラメラの数が多く，その部分に特別に現われいわば挿入されたようになっている．またラメラの厚さは一様でなく多数の小結節様肥厚が認められる．グラナが全く認められないのは特に注意すべきでストローマもまた Peristromium 以外には

でんぷん粒の周辺にいわゆる挿入されたラメラの末端との間に僅かに介在するにすぎない．更に特殊なものとしてレンズ状小体がラメラ間に介在し特に周辺部には多数現われ 0.5μ〜$1.5\mu \times 0.04$〜0.06μ 位で内部は 30〜50 Å の糸状要素が不規則に密に詰まりツノゴケのピレノイドに近似している．光顕で従来認められていたグラナの電顕的同定はなお将来の問題として残される．　　（吉田吉男）

植物社会学(群落学)文献国際雑誌

Tüxen, R., Excerpta Botanica Sectio B Sociologica **1**: (1) 1-87, (2) 89-191, (3) 193-264, (4) 265-319 (1959).

1957 年 Pavia（北イタリア）で開かれた国際植生学会会議 (Tagung der Internationalen Vereinigung für Vegetationskunde) での要望により植物社会学文献雑誌の発刊を見るにいたった．本誌の編集委員会は各国の代表的植物群落学者40数人により構成され，その責任主筆として同学会事務局長 Prof. Dr. Dr. h. c. R. Tüxen（ドイツ国立植生地図研究所長）が当っている．本誌は現代の植物社会学に関する世界中の文献を完全に収録することを目的とし，毎年 1 巻（1-4 号）ずつ発行されることになっている．

内容の配列は研究された国別（研究者の所属国とは無関係）および研究題目・内容別の 2 本建てとされている．DK システムにより植物社会学の全分野に亘って項目別に配別されている．主な項目として，植物社会学の歴史・術語・群落形態学・群落生態学・遷移・群落の歴史・群落の分布・群落分類学・応用植物社会学・隣接科学との境界領域などが挙げられる．

現在までに第 1 巻 4 号まで出版されている．第 1 号は国別文献目録で，日本（1959 年まで 443 篇）および R. Tüxen と H. Meissner によるドイツ (1956-1958 年まで 568 篇）を収録している．第 2 号は項目別に群落学上の問題に言及したフロラについて O. de Bolòs 以下 14 人より，および B. Grosse と R. Tüxen による群落学と土壌学に関係した各国の文献が収められている．さらに国別では A. Borza によるルーマニアの文献リストが記載されている．第 3 号には，国別では H.C. Hanson によるアラスカ・M. Zohary によるパレスチナ・L. Reichling によるルクセンブルグの文献，項目別では O. Wilmanns による特定群落の根系を研究したもの(各国)・R. Tüxen による植物群落の分布と地域図を扱かったもの（主として本号はヨーロッパ）が収録されている．第 4 号は Erika et Sandro Pignatti によるイタリヤに関した 1900-1959 年までにおよぶ 593 篇が収められている．

各掲載文献は，著者名・雑誌名・巻号・頁数・年号はもちろん雑誌・著書の発行地名まですべて記載されている．数年後には，名実共に斯学に関する世界の文献を完全に網羅した文献集となるものと期待されている．現在のところ各文献の内容の紹介は予定されていない．Stuttgart の Verlag Gustav Fischer から出版されており．各巻 30 DM. 国際植生学会または Floristisch-soziologische Arbeitsgemeinschft の会員は，出版社から直接購入する場合に限り各巻 24 DM. となっている．

（附記）第 1 巻 1 号の国別文献集日本篇は，紹介者が短時日の中に主として関係研究者から好意的に届いたリストによって行なった．ヨーロッパでは，今まで比較的この分野の日本の研究が知られていなかったため，わが国の研究者による今までの業績はかなりの反響を呼んでいる．1958 年度までの記載漏れ，および 1959 年以降の植物群落学に関係した論文・著書のリストまたは別刷を紹介者あてに送って戴けば，逐次まとめて加えてゆきたい．日本の文献をより完全に収録するため御協力をお願いします．　　（宮脇　昭）

日本植物学会第25回大会

プログラム

学会会長 服部静夫

大会会長 三木茂

昭和35年11月2日(水)—4日(金)

大阪大学医学部・理学部

大阪 1960

日　　程

		9–12	12–13	13–18	夜
2日（水）	講演番号	1〜10	昼食	11〜25	分類学会
	A 会場	生理・生化		生理・生化	
	B 〃	生理・生化		生理・生化	
	C 〃	分類・地理		分類・形態・地理	
	D 〃	形態		細胞・遺伝	
	E 〃	生態		生態	
3日（木）	講演番号	26〜35	記念撮影・昼食	総会（講堂）／シンポジウム	光合成研究者懇談会 細胞微細構造懇談会 藻類学会 生態の集まり 酵母研究者の集まり
	A 会場	生理・生化			
	B 〃	生理・生化			
	C 〃	形態・分類・地理		2. 細胞の増殖と分化	
	D 〃	細胞			
	E 〃	生態・形態		3. 日本における遷移	
4日（金）	講演番号	36〜45	昼食	シンポジウム	生理談話会
	A 会場	生理・生化		4. 微生物の代謝（講堂）	
	B 〃	生理・生化			
	C 〃	分類・地理・生理		1. 植物の系統	
	D 〃	細胞			
	E 〃				

大会前日（11月1日）の行事

§ **日本シダ学会**　9〜15 時　　大阪市立自然科学博物館（大阪市西区靱中通2丁目）

§ **酵母に関するシンポジウム**　13〜17 時　日本菌学会・酵母研究会共催

大阪大学理学部（大阪市北区中之島）大講堂

§ **植物学研究連絡委員会**　16〜17.30 時　　大阪大学理学部会議室

§ **評議員会**　17.30〜21 時　　大阪大学理学部会議室

会期中のその他の行事

§ **光合成研究者懇談会**　3日　18〜22 時　　大阪大学内の予定

§ **酵母研究者の集まり**　3日　18.30 時　　中央公会堂（中之島公園）地下食堂

大会翌日（11月5日）の行事

§ **近郊見学**　　9時大阪駅前集合　日帰り

§ **公開講演**　　13〜17 時　　朝日新聞大阪本社（大阪市北区中之島）講堂

日本植物学会主催・大阪府高等学校生物教育研究会・大阪市立自然科学博物館・朝日新聞社後援

浜田　稔　マツタケの科学―生長と発生予察

木原　均　シッキムとアッサムの稲採集旅行談（スライドおよびテープ使用）

なお，11月6日には，日本自然保護協会主催の **'自然保護に関するシンポジウム'** が開かれる予定です．13〜17 時，朝日新聞大阪本社講堂

A 会場〔生理・生化〕

A 1	9.00―9.12	増田芳雄	大阪市大・理	オーキシン作用による細胞壁ゆるみとカルシウムの関与
A 2	9.15―9.27	依田静子* 芦田譲治	京大・理 〃	IAA の浸透価におよぼす影響
A 3	9.30―9.42	猪狩盛夫* 長尾昌之	東北大・理 〃	根におけるインドール酢酸の吸収ならびに生体内変化
A 4	9.45―9.57	長尾昌之* 斎藤周夫 笹岡信	東北大・理 〃 〃	シュウカイドウ種子の発芽におけるジベレリンと光の作用
A 5	10.00―10.12	和田清美	静岡大・文理	小麦，大根の無菌培養におけるジベレリンおよびインドール酢酸の効果について
	10.15―10.30	〔総合討論〕		
A 6	10.30―10.42	村上浩	農技研	種子の登熟および発芽過程におけるジベレリンの消長
A 7	10.45―10.57	勝見允行	国際基督大	黄化エンドウ茎切片に対するカイネチンの影響 II
A 8	11.00―11.12	山田妙子* 橋本徹 師尾武子 高橋憲子 八巻敏雄	日本女子大 東大教養・武蔵高校 日本女子大 〃 東大・教養	タバコ種子の GA による発芽におよぼす無機塩類の影響
A 9	11.15―11.27	師尾武子* 橋本徹 山田妙子 高橋憲子 八巻敏雄	日本女子大 東大教養・武蔵高校 日本女子大 〃 東大・教養	タバコ種子の GA による発芽におよぼす有機酸塩類の影響
A10	11.30―11.42	橋本徹* 師尾武子 山田妙子 高橋憲子 八巻敏雄	東大教養・武蔵高校 日本女子大 〃 〃 東大・教養	タバコ種子の発芽におよぼす燐酸イオンの影響
	11.45―12.00	〔総合討論〕		

B 会場〔生理・生化〕

B 1	9.00―9.12	瀬野悍二* 芦田譲治	京大・理 〃	酵母の銅訓養中における遺伝的変化（II）
B 2	9.15―9.27	村山徹郎* 芦田譲治	愛媛大・文理 京大・理	銅耐性酵母の有機酸およびアミノ酸代謝 III
B 3	9.30―9.42	宮本典子* 芦田譲治	京大・理 〃	酵母菌物質代謝の銅阻害
B 4	9.45―9.57	中村運	甲南大・理	酵母のカドミウム耐性における核酸および蛋白質代謝の意義
B 5	10.00―10.12	森下日出旗* 奥田正男 高田英夫	大阪市大・理 〃 〃	ポリエチレン高張環境におけるコーボの生理
	10.15―10.30	〔総合討論〕		
B 6	10.30―10.42	山本武* 高田英夫	大阪市大・理 〃	ストロンチューム高張環境によるコーボ裸プロトプラストの形成と再生
B 7	10.45―10.57	柳島直彦	大阪市大・理	酵母の呼吸系欠損変異とオーキシン

B 8	11.00—11.12	永井　進	大阪市大・理	コーボの不安定な菌株について
B 9	11.15—11.27	西上一義	島根大・文理	酵母の呼吸の比較生理 glucose, gluconate, pyruvate, arabinose, ethyl alcohol の酸化について
B10	11.30—11.42	奥田慎一	東北大・農	野生酵母 *Hansenula saturnus* の胞子形成
B10′	11.45—12.00	倉石　衍	東北大・農	酵母のパントテン酸欠乏による死細胞出現

C 会 場 (分類・地理)

C 1	9.00—9.12	堀川芳雄 関太郎*	広島大・理 〃	*Brotherella henoni* (Duby) Fl. カガミゴケについて
C 2	9.15—9.27	髙木典雄	名大・教養	中部高山地域における Dicranaceae (シッポゴケ科) 蘚類の分布
C 3	9.30—9.42	新　敏夫	鹿児島大・文理	日本産ツヤゴケ科蘚類の研究，ツヤゴケ属について (2)
C 4	9.45—9.57	井上　浩	教育大・理	ゼニゴケ目植物の胞子発芽，特に第一次仮根形成の型
C 5	10.00—10.12	川崎次男	東京学芸大	ウラボシ科の胞子について
	10.15—10.30	〔総合討論〕		
C 6	10.30—10.42	椿　啓介	長尾研究所	南極採集品から分離した糸状菌について
C 7	10.45—10.57	曽根田正己	長尾研究所	南極土壌より分離せる酵母菌について
C 8	11.00—11.12	信夫隆治* 川戸峯子	大阪学芸大	青緑水溶性色素を生産する放線菌の一新種 *Streptomyces indigoferus* について
C 9	11.15—11.27	寺川博典	東京医歯大	高等菌類の分離と再生
C10	11.30—11.42	佐藤正己	茨城大・文理	螢光分析法によるムシゴケ属地衣の分類
	11.45—12.00	〔総合討論〕		

D 会 場 (形 態)

D 1	9.00—9.12	板垣史郎	協和醱酵	*Micrococcus glutamicus* の細胞学的研究(第5報). 有機酸の形態におよぼす影響—主として branching について
D 2	9.15—9.27	村岡節雄* 野口彰	熊本大・理 〃	ヨツバゴケ類の無性芽形成と発芽
D 3	9.30—9.42	髙橋千裕	名大・教養	シダ配偶体の暗培養
D 4	9.45—9.57	小野記彦* 榎本雅敏	都大・理	ミジンコウキクサにみられる老化とその回復現象
D 5	10.00—10.12	岩崎尚彦	都大・理	シャジクモ科植物の生長点の分化と器官形成. IV. *Chara Braunii.*
	10.15—10.30	〔総合討論〕		
D 6	10.30—10.42	吉田　治	千葉大・文理	フウトウカズラの胚のう形成についての二・三の考察
D 7	10.45—10.57	及川公平	三重大・学芸	アマナの胚嚢について
D 8	11.00—11.12	福本日陽	東京農工大・教養	*Bryophyllum* の不定芽形成について
D 9	11.15—11.27	原　襄	東大・教養	オニシバリの生長点構造
D10	11.30—11.42	熊沢正夫	名大・教養	ハマオモト属における分枝法
	11.45—12.00	〔総合討論〕		

E 会場〔生態〕

E 1	9.00—9.12	南川　幸	菰野高	鈴鹿山脈中北部の植物群落
E 2	9.15—9.27	横川広美	鳥取大・学芸	山陰地方のアカマツ林について
E 3	9.30—9.42	秋武和俊* 鴨川　誠 細川隆英	九大・理 〃 〃	桜島の植生構造．I．植生一般及び熔岩について
E 4	9.45—9.57	小村　精* 宮田逸夫	九大・理 〃	桜島の植生構造．II．類似度からみた各熔岩上の植生
E 5	10.00—10.12	田川日出夫* 小谷信矢	九大・理 〃	桜島の植生構造．III．熔岩上の植物の分布パタンと群集の分岐度
	10.15—10.30	〔総合討論〕		
E 6	10.30—10.42	野本宣夫* 佐伯敏郎 門司正三	茨城大・文理 東大・理 〃	高等植物の葉の光・同化曲線
E 7	10.45—10.57	楠元　司	鹿児島大・教育	沿海地ならびに高地の常緑広葉樹の光合成および呼吸能力
E 8	11.00—11.12	佐伯敏郎	東大・理	葉の生長におよぼす物質生産の影響
E 9	11.15—11.27	高田和男 黒岩澄雄* 広井敏男 萩元育夫	取手一高 東大・理 〃 酒田東高	異なつた相対照度下における生長の解析
E10	11.30—11.42	高田和男* 黒岩澄雄 岩城英夫	取手一高 東大・理 〃	巣まき植物の生長におよぼす巣内個体密度の影響
	11.45—12.00	〔総合討論〕		

A 会場〔生理・生化〕

A11	13.00—13.12	柴岡弘郎* 八巻敏雄	東大・教養 〃	ヒマワリ属の葉に含まれる生長阻害物質
A12	13.15—13.27	林　克己	広島大・理	トウキビの芽生えにおいてアミノ酸の組成におよぼす生長ホルモンの影響
A13	13.30—13.42	小西通夫	京大・農	ナフテン酸の根の生長促進作用について
A14	13.45—13.57	萩本　宏* 小西通夫	京大・農 〃	担子菌子実体の生長を促進する作用物質の研究．V．ツクリタケ（西洋マツタケ）子実体の生長ホルモンとしてのアミノ酸
A15	14.00—14.12	丸重啓二* 丸重靖子	京大・農 〃	アサガオの芽生における日長感受性の成立と代謝
A16	14.15—14.27	片山忠夫	京大・農	稲属各種の感光性の研究
A17	14.30—14.42	藤伊　正* 石川茂雄	教育大・理 〃	種子の発芽における短日的光週性
A18	14.45—14.57	中山　包 白木健助*	信州大・文理 〃	サボンソウの種子発芽に対する温度と光の影響
	15.00—15.15	〔総合討論〕		
A19	15.15—15.27	河原　晨	大阪市大・理	ヒシモドキの発芽について
A20	15.30—15.42	嚴佐耕三* 生物研究会	阪大・教養	花粉の発芽に対する無機物，特に硼素の発芽に対する効果
A21	15.45—15.57	市村国彦* 井沢三生 太田行人	名大・理 〃 〃	発芽種子の可溶性 RNA

A22	16.00—16.12	堀　武義	岐阜大・学芸	マツバボタンの開花閉花に伴なう各器官における生長素の消長
A23	16.15—16.27	堀江格郎	兵庫農大	ムラサキツユクサの花の開閉に関係する要因
A24	16.30—16.42	加藤勇夫	広島大・教養	気孔発生過程の表皮組織におけるフォスフォリラーゼ作用
A25	16.45—16.57	相馬悌介	新潟大・教育	気孔の開閉運動に対する通気の影響について
	17.00—17.15	〔総合討論〕		

B 会場 〔生理・生化〕

B11	13.00—13.12	山田晃弘	東大・教養	トウゴマ発芽時におけるグリセリン代謝と脂肪酸代謝との関係
B12	13.15—13.27	辻　英夫* 浜田秀男	兵庫農大 〃	イネおよびエンバクの発芽に伴なう芽生中の酸溶性燐酸エステル量（特にイネの $\varDelta 7P$ 量）の変化
B13	13.30—13.42	石川　鉱	北大・理	秋まきコムギ胚の低温処理中における物質代謝系の変動，特に核酸の代謝について
B14	13.45—13.57	杉村康知* 薬師寺英次郎	東邦大・理	数種の海藻のチトクロームについて
B15	14.00—14.12	今関英雅* 山本時彦 瓜谷郁三	名大・農 〃 〃	オオカメノキの葉の二糖配糖体加水分解酵素
B16	14.15—14.27	安田　齊	信州大・文理	白バラ花弁に対するロイコアントチアン反応
B17	14.30—14.42	遠藤　徹	遺伝研	パンジーその他の青色花および紫色花のアントシアニン
B18	14.45—14.57	林　孝三 斎藤規夫* 三井清司	教育大・理 〃 〃	ヤグルマギク青色花のメタロアントシアニンについて
	15.00—15.15	〔総合討論〕		
B19	15.15—15.27	桑名　誉	阪大・理	アカパンカビの高温感受性致死突然変異株の遺伝生化学的研究
B20	15.310—5.42	佐々木喜美子	北大・理	ベゴニヤの酸化能に関する二・三の知見
B21	15.45—15.57	笠巻明子* 佐々木昭治 宇佐美正一郎	北大・理 〃 〃	*Proteus vulgaris* による嫌気的条件におけるコハク酸生成機作について
B22	16.00—16.12	和気和民* 宇佐美正一郎	北大・理 〃	クロカビの分生胞子形成期におけるいくつかの知見
B23	16.15—16.27	鈴木　昇* 奥田　聰 鈴木　旺	愛知女子大 名大・教養 〃	*Azotobacter* の窒素代謝
B24	16.30—16.42	井上行雄* 久保秀雄	科研化学 〃	*Streptomyces griseus* の物質代謝. IX. Intact mycelium によるアミノ酸々化についての研究. Part 1. 培養各期のアミノ酸々化の概要
B25	16.45—16.57	井上行雄* 久保秀雄	科研化学 〃	Part 2. Intact mycelium によるモノアミノモノカルボン酸の酸化
	17.00—17.15	〔総合討論〕		

C 会場 〔分類・地理・形態〕

C11	13.00—13.12	川戸峰子* 信夫隆治	大阪学芸大 〃	放線菌の窒素源利用について. I. NO_3 および NO_2 について

C12	13.15—13.27	砂山真理子	東邦大・理	養鰻池における水生菌類および幼鰻に対する感染実験
C13	13.30—13.42	増田染一郎	三生製薬研	Myxobacteria の水中子実体について
C14	13.45—13.57	小林艶子	横浜市大・文理	南極産ケイソウ *Navicula muticopsis* V. Heurk の変異
C15	14.00—14.12	福島博	横浜市大・文理	南氷洋の着色氷の生物学的研究
C16	14.15—14.27	根来健一郎	京大・理	南氷洋の浮氷を彩どる藻類
C17	14.30—14.42	熊野茂* 瀬戸良三 広瀬弘幸	神戸大・理 神戸女学院 神戸大・理	カワモズク科数種の嚢果形成過程の比較
C18	14.45—14.57	瀬戸良三* 熊野茂 広瀬弘幸	神戸女学院 神戸大・理 〃	再びカワモズク属の Chantransia stage について
	15.00—15.15	〔総合討論〕		
C19	15.15—15.27	丸山晃	横浜市大・文理	北海道東北海岸湖沼のコッコイド・ランソウ相
C20	15.30—15.42	猪野俊平* 熊谷信孝 石井慶三	岡山大・理 〃 〃	アミジグサ目の形態発生．I，II．アミジグサ目数種の四分胞子嚢形成と成熟分裂
C21	15.45—15.57	西林長朗* 猪野俊平	岡山大・理 〃	コンブ目の形態発生学的研究．IV, V, VI．二三コンブ目植物の遊走子嚢発生と遊走子形成
C22	16.00—16.12	桃谷好英	京大・理	蛋白質から見たカエデ属の類縁について
C23	16.15—16.27	藤田安二	大工試	ナギナタコウジュ，タイワンナギナタコウジュおよびフトボナギナタコウジュ
C24	16.30—16.42	三木茂* 粉川昭平	大阪市大・理 〃	九州の遺体植物
C25	16.45—16.57	堀川芳雄	広島大・理	本邦における熱帯性植物の北上分布
	17.00—17.15	〔総合討論〕		

D 会 場 〔細胞・遺伝〕

D11	13.00—13.12	佐々木正人	立教大	車軸藻類における染色体数
D12	13.15—13.27	益森静生	山口大・教育	*Artemisia* 属数種における細胞学的研究
D13	13.30—13.42	荒野久雄	昭和薬大	邦産キク亜科における核型，特に一属一種の種について
D14	13.45—13.57	佐藤重平	東大・教養	ショウガ目植物の原始核型と安定核型
D15	14.00—14.12	神野太郎	愛媛大・教育	*Rubus Nishimuranus* Koidz. の子孫の細胞遺伝学的研究
D16	14.15—14.27	西岡泰三	都大・理	ニガナ類植物の分化についての 2・3 の知見
D17	14.30—14.42	浅野明	栄光学園	海岸植物の核学的研究，第4報
D18	14.45—14.57	辰野誠次	広島大・理	ケゼニゴケの倍数性とその本邦ならびに近域における分布について
	15.00—15.15	〔総合討論〕		
D19	15.15—15.27	稲荷山資生 竹村英一*	奈良学芸大 教育大・理	ヒガンバナ属の人工雑種について（第4報）
D20	15.30—15.42	増淵法之	北大・理	小麦における枝穂の遺伝学的研究
D21	15.45—15.57	福田一郎	東京女子大	オオバナノエンレイソウの自然集団に現われた染色体異常

D22	16.00—16.12	木村劼二* 藤生みさ子	岡山大・理 〃	ウシグソヒトヨの不開傘子実体
D23	16.15—16.27	竹中要	遺伝研	進化の一例証
D24	16.30—16.42	佐藤進一	弘前大・文理	ムラサキツユクサ倍数種の DNA (Feulgen) 含有量について
D25	16.45—16.57	松浦一 岩渕雅樹*	北大・理 〃	細胞分裂におよぼす塩類の影響. I. NaCl 処理による *Tradescantia* PMC の分裂異常
	17.00—17.15	〔総合討論〕		

E 会場〔生態〕

E11	13.00—13.12	鈴木時夫	大分大・学芸	モミーシキミ群集について
E12	13.15—13.27	矢野悟道	広島大・理	植物地下器官の生態学的研究: 砂丘植物について
E13	13.30—13.42	笠原安夫	岡山大・農	耕地雑草群落の構造（I）
E14	13.45—13.57	森千春		雑草遷移に関する考察（3）
E15	14.00—14.12	飯泉茂* 菅原亀悦	東北大・理 岩ヶ崎高	ウマタテバにおける再植生過程
E16	14.15—14.27	辻井達一 小島覚*	北大・農 〃	鹿部放牧地のドクゼリ集落
E17	14.30—14.42	菅沼孝之* 井上敬子 小清水卓二	奈良女子大・理 〃 〃	奈良若草山の植物群落. 3. ススキートダシバ群落の季節的遷移について
E18	14.45—14.57	岩城英夫* 翠川文次郎	東大・理 都大・理	霧ヶ峯草原における各種群落の分布について
	15.00—15.15	〔総合討論〕		
E19	15.15—15.27	二村坦孝	教育大・理	湖沼における二・三の水生不完全菌類の分布の特性について
E20	15.30—15.42	鈴木静夫	東京理大・薬	湖底泥における水生菌類の分布と生態
E21	15.45—15.57	今堀宏三* 須賀瑛文	阪大・教養 あずま中	湖沼における Charetum の遷移
E22	16.00—16.12	坂本充* 西条八束	名大・理 〃	冬期湖沼における植物プランクトンの物質生産について
E23	16.15—16.27	市村俊英* 有賀祐勝	教育大・理 東大・理	海洋植物プランクトンの光合成特性と基礎生産における意義
E24	16.30—16.42	宝月欣二* 岡西良治 菅原久枝	都大・理 城南小 小野学園	植物性プランクトンの生長におよぼす水草の影響
E25	16.45—16.57	塚田松雄	大阪市大・理	後氷期の花粉分析的研究. V. 最終晩氷期以後の花粉分布図
	17.00—17.15	〔総合討論〕		

A 会場〔生理・生化〕

A26	9.00—9.12	上坪英治	阪大・理	カナダモの原形質流動におよぼす光の影響
A27	9.15—9.27	山段忠	京都学芸大	フラスモ細胞におよぼすキレート剤の影響
A28	9.30—9.42	田沢仁	阪大・理	湿室中に置いたフラスモの浸透価におよぼす光の影響

A 29	9.45—9.57	永井怜子* 田沢仁	阪大・理 〃	フラスモの浸透調節における膜電位の役割
A 30	10.00—10.12	小田健二	東北大・理	シャジクモの膜電位とイオン分布
	10.15—10.30	〔総合討論〕		
A 31	10.30—10.42	柴岡孝雄	東北大・理	微小電極法によるオジギソウ活動電位の研究
A 32	10.45—10.57	須田省三	神戸大・理	オジギソウの抗興奮性物質について
A 33	11.00—11.12	鳥山英雄	東京女子大・理	オジギソウの細胞生理学的研究（第 12 報）—紐状装置の運動現象
A 34	11.15—11.27	鳥山英雄	東京女子大・理	オジギソウの細胞生理学的研究（第 13 報）—昼夜によつて形態的変化を示す小体について
A 35	11.30—11.42	遠藤沖吉	宮城農短大	光遮断効果の Latency について
	11.45—12.00	〔総合討論〕		

B 会 場 〔生 理・生 化〕

B 26	9.00—9.12	松下亀久	九大・理	TMV の増殖に関する研究
B 27	9.15—9.27	牧野利一	札幌医大	サルモネラ菌によるクエン酸の代謝
B 28	9.30—9.42	香山時彦	和歌山大・学芸	細菌多糖類 K. C. G. の制ガン作用（第 1 報）
B 29	9.45—9.57	伊藤太郎	帯広畜産大	アカパンカビの誘引性ホルモン様物質の分離
B 30	10.00—10.12	宮本義男	愛媛大・文理	微生物によるパラフィンおよびろうの分解. その一過程
	10.15—10.30	〔総合討論〕		
B 31	10.30—10.42	大槻虎男	お茶の水大・理	好稠糸状菌の研究
B 32	10.45—10.57	西尾隆昌	広島大・理	抗酸菌の呼吸におよぼす滲透圧の影響について
B 33	11.00—11.12	賀来章輔	下関商高	植物組織の凍結曲線の分析（Ⅲ）
B 34	11.15—11.27	畠山伊佐男	京大・理	緑葉の生・死組織の氷点の差異—生体膠質結合水の示唆
B 35	11.30—11.42	畠山伊佐男 河野清*	京大・理 京工繊大	吸水力におよぼす外液の界面張力の影響
	11.45—12.00	〔総合討論〕		

C 会 場 〔形態・分類・地理〕

C 26	9.00—9.12	上野実朗	大阪市大・理	裸子植物花粉の形態
C 27	9.15—9.27	原田賢之 村上道夫 竹岡政治*	京都府大・農 〃 〃	禾本科植物花粉の表面微細構造
C 28	9.30—9.42	幾瀬マサ	東邦大・薬	蜜蜂の花粉だんご (Pollen loads) の検定
C 29	9.45—9.57	加藤一男* 渡辺光太郎	京大・理 京大・農	イネ科以外の他科植物における柱頭反応
C 30	10.00—10.12	塙順	都大・理	葉の脊腹性についての一実験
	10.15—10.30	〔総合討論〕		
C 31	10.30—10.42	秋山優	島根大・文理	本邦産土表性藻類 *Fritschiella* の生態
C 32	10.45—10.57	野田光蔵	新潟大・理	佐渡海峡の海藻
C 33	11.00—11.12	新崎盛敏* 徳田広	東大・農 〃	東京湾産ヒトエグサの生活史, 特に *Gomotia* 型発芽体について

C 34	11.15—11.27	長谷川 由 雄	北海道水産研	ミツイシコンブの生態学的研究．II．生活史について
C 35	11.30—11.42	田 中 剛* 野 沢 洽 治	鹿児島大・水 〃	南西諸島産ウミウチワ属について
	11.45—12.00	〔総 合 討 論〕		

D 会 場 〔細 胞〕

D 26	9.00—9.12	三 木 寿 子	京 大・理	キカノコユリの子房内における物質交代
D 27	9.15—9.27	山 岸 秀 夫	京 大・理	螢光染色法による細胞内微小カ粒の分類
D 28	9.30—9.42	伊 倉 伊三美	山形大・教育	シダ類精子の生存時間や形態におよぼす精子毒の作用
D 29	9.45—9.57	土井田 幸 郎	遺 伝 研	タデ属植物の発生学的研究．IV．タデ属数種の花芽形成および花粉粒形成におよぼす生長物質の効果
D 30	10.00—10.12	川 松 重 信	愛知学芸大	アカウキクサ (*Azolla imbricata* Nakai) の根の電子顕微鏡的観察
	10.15—10.30	〔総 合 討 論〕		
D 31	10.30—10.42	左 貝 アイ子	奈良女子大・理	植物細胞のオスミウム固定についての電子顕微鏡的研究．III．pH の影響について
D 32	10.45—10.57	重 永 道 夫	奈良女子大・理	花粉母細胞で得られた電顕像について
D 33	11.00—11.12	神 谷 平	愛知学芸大	接合藻類の電子顕微鏡観察
D 34	11.15—11.27	丸 山 圭 蔵	京 大・理	葉緑素欠損植物の葉緑体の電子顕微鏡的研究
D 35	11.30—11.42	新 家 浪 雄	京 大・理	*Volvox* 細胞の電子顕微鏡的構造
	11.45—12.00	〔総 合 討 論〕		

E 会 場 〔生 態・形 態〕

E 26	9.00—9.12	倉 内 一 二	豊橋東高	伊勢湾台風の害と回復状況——塩風害と海岸林 II
E 27	9.15—9.27	高 橋 基 生	東 大・理	春化処理の根系呼吸におよぼす影響
E 28	9.30—9.42	高 橋 基 生	東 大・理	特異分布ならびに生育に対する生態学的研究第1報．御蔵島における寒地植物と暖地植物の共存現象
E 29	9.45—9.57	堀 川 芳 雄 岡 本 香*	広島大・理 〃	広島県産スゲ属植物について
E 30	10.00—10.12	鈴 木 貞 雄	宇都宮中央女子高	チマキザサ類 *Sasa* sect. *Eusasa* の葉の隅どりの生態
	10.15—10.30	〔総 合 討 論〕		
E 31	10.30—10.42	高 橋 茂 生		北海道仁山におけるエンレイソウ属植物の生態について
E 32	10.45—10.57	鴨 川 誠* 秋 武 和 俊	九 大・理 〃	ヤドリギ類の宿主選択について——おもに植付実験について
E 33	11.00—11.12	吉 良 竜 夫* 庵 原 遜	大阪市大・理 〃・植物園	若いクロマツ人工林の蒸発散量
	11.15—11.30	〔総 合 討 論〕		
E 34	11.30—11.42	木 原 均 末 本 雛 子*	遺 伝 研 京 大・農	一粒系コムギにおける左右性の発現機構について
E 35	11.45—12.00	木 原 均	遺 伝 研	左右性の決定に関する考察

シンポジウム

話題 2　細胞の増殖と分化　(C 会場)

14.30—15.05	高尾昭夫	名大・理	胚の発生と物質の貯蔵過程
15.15—15.50	中沢信午	山形大・文理	分化における皮部細胞質の役割
16.00—16.35	森本　孝	大阪医大・生理	ユーグレナの呼吸阻害と細胞分裂
16.45—17.30	〔総合討論〕		

話題 3　日本における遷移　(E 会場)

14.30—15.05	手塚泰彦	資源研	遷移の機構——土壌要因の役割を中心に
15.15—15.50	沼田　真	千葉大・文理	二次遷移と遷移診断
16.00—16.35	吉岡邦二	東北大・理	わが国の火山における一次遷移
16.45—17.30	〔総合討論〕		

A 会場〔生理・生化〕

A36	9.00—9.12	渡会彰彦	北大・理	葉緑体成分分子比と光合成単位
A37	9.15—9.27	西田晃二郎	金沢大・理	光照射下に葉から排出される $C^{14}O_2$ について
A38	9.30—9.42	藤茂宏* 佐藤公行	岡山大・理 〃	緑色植物の光化学的亜硝酸還元系の生化学的研究（その 3）
A39	9.45—9.57	桜井英博	東大・理	キャベツ白葉の緑化に伴なう光合成に関連した機能および物質の発達について
A40	10.00—10.12	伊沢清吉	東大・理	葉緑体におけるビタミン K_3（メナジオン）の酸化還元とヒル反応
	10.15—10.30	〔総合討論〕		
A41	10.30—10.42	加藤　栄	東大・理	クロレラより抽出した二三の酸化還元蛋白について
A42	10.45—10.57	千葉保胤* 菅原　淳 佐々木　弘	九大・理 〃 〃	アルミナ処理をした葉緑体の photophosphorylation
A43	11.00—11.12	菅原　淳	九大・理	葉緑体への P^{32} のとりこみ．II．各分画の specific activity
A44	11.15—11.27	宮地重遠	東大・応微研 徳川生研	クロレラの細胞内における核酸および燐蛋白質への燐酸導入機作に関する研究
A45	11.30—11.42	服部明彦 藤田善彦*	東大・応微研 〃	フィコビリン暗生成過程における細胞内物質の変動
	11.45—12.00	〔総合討論〕		

B 会場〔生理・生化〕

B36	9.00—9.12	稲葉耕三	広島大・理	植物の窒素代謝におよぼすカリウムの影響．(II)．RNA への P^{32} の組み入れについて
B37	9.15—9.27	本田　稔	広島大・理	水分欠乏植物の光合成速度と呼吸量について
B38	9.30—9.42	高沖　武	広島大・理	水分欠乏植物における 2・3 の酵素活性について（第 2 報）
B39	9.45—9.57	相見霊三	農技研	高等植物（イネ）体内における酸素の積極的輸送機能について

B40	10.00—10.12	山本昌木	島根農大	*Phytophthora infestans* (Mont.) DeBary 系統の菌体成分と病原性について
	10.15—10.30	〔総合討論〕		
B41	10.30—10.42	岡本　尚	名大・理	好塩性クラミドモーナスの細胞構造に対する種々の溶質の作用
B42	10.45—10.57	藤井良平	名大・理	ウキクサの休眠体の形成
B43	11.00—11.12	菅井道三	名大・理	シダ配偶体の造精器形成因子について
	11.15—11.30	〔総合討論〕		

C 会 場 〔分類・地理・生理〕

C36	9.00—9.12	豊国秀夫	北大・理	ユウバリリンドウの群
C37	9.15—9.27	小山鉄夫	東大・理	サルトリイバラ属の分類
C38	9.30—9.42	鈴木昌友	茨城大・文理	東北地方南部のカシワバハグマ属植物
C39	9.45—9.57	原田市太郎	名大・理	セキショウモ属の核型
C40	10.00—10.12	浜田秀男	兵庫農大	印度支那稲の分類と分布地域
	10.15—10.30	〔総合討論〕		
C41	10.30—10.42	西村公臣	奈良女子大・理	ゴフンゴケから分離された藻の培養
C42	10.45—10.57	尾形英二	水産講習所	コンコセリス糸状体の生長と炭酸源について
C43	11.00—11.12	入来義彦 三輪知雄*	教育大・理 〃	緑藻細胞膜間粘質物の生化学的研究．I．ヒトエグサ (*Monostroma*) の細胞膜間質
C44	11.15—11.27	福田育二郎* 児玉公一	東京理大 〃	藍藻細胞の同調培養による生理学的研究．その 1．窒素代謝について
	11.30—11.45	〔総合討論〕		

D 会 場 〔細 胞〕

D36	9.00—9.12	松浦一 谷藤茂行* 岩淵雅樹	北大・理 〃 〃	X線照射による染色体異常頻度におよぼす二三化学物質の影響
D37	9.15—9.27	松浦一	北大・理	染色体基質の構造について
D38	9.30—9.42	湯浅明	東大・教養	コウボキンの紡錘体について
D39	9.45—9.57	島村環* 太田敬久	名大・理 〃	核分裂機構の映画による解析
D40	10.00—10.12	吉田吉男	新潟大・理	二三の細胞質活性におよぼす核の相関性
	10.15—10.30	〔総合討論〕		
D41	10.30—10.42	武久慎	北大・理	ソラマメの体細胞染色体におよぼす EDTA 処理の効果
D42	10.45—10.57	石田政弘	京大・理	*Marchantia polymorpha* の核酸について
D43	11.00—11.12	横村英一	京大・理	*Vicia faba* の根端細胞核の DNA 量について
D44	11.15—11.27	下斗米直昌* 進藤公夫 田羅征伸	広島大・理 〃 〃	天然属間雑種ノコンギク×オオユウガギクの細胞学的研究
D45	11.30—11.42	大野林二郎	北大・理	トウガラシとシシトウガラシの正逆接木にみられる種々なる変化について
	11.45—12.00	〔総合討論〕		

シ ン ポ ジ ウ ム

話 題 1　植 物 の 系 統　(C 会 場)

13.00—13.30	加崎英男	都 大・理	生殖器官の形質から見た見解
13.40—14.10	瀬川宗吉	九 大・農	生活環の型式から見た見解
14.20—14.50	植田利喜造	教育大・理	生理学的形質から見た見解
15.00—15.30	三木 茂	大阪市大・理	古生物上の二三の例証
15.40—16.30	〔総 合 討 論〕		

話 題 4　微 生 物 の 代 謝　(講 堂)

13.00—17.00	岩塚 寿*・丸山 槇子 久野 光造・森 健志	名 大・理	硫黄細菌の CO_2 固定
	金井 竜二*・宮地 重遠 高宮 篤	東 大・理	微生物における酸水素反応と共役した炭酸固定
	尾形 昭逸	北 大・農	光合成細菌の Photosynthesis と Chemosynthesis. *Chromatium* sp. の Photophosphorylation, Pyridine Nucleotide Reduction と Carbon Assimilation Pathway
	森 健志*・岩崎 秀一 大西 智子・鈴木 秀穂	名 大・理	細菌の脱窒素反応

会 場 へ の 交 通 案 内 (大阪駅より)

大阪大学医・理学部 (大会々場)

	系統番号	行 先	下車場所
市 電	(1)	大国町・阿倍野	肥後橋
	(4)	難波・阿倍野	〃
	(10)	肥後橋	〃
	(13)	福島・桜川	出入橋
	(15)	難波	肥後橋
	(22)	港車庫	〃
市バス	(45)	大阪港	田蓑橋
	(ト45)	第3突堤	〃
	(53)	辰已橋	〃

徒歩では大阪駅西口より 15 分

市立自然科学博物館 (シダ学会々場)

	系統番号	行 先	下車場所
市 電	(1)	大国町・阿倍野	靱(ウツボ)公園前
	(4)	難波・阿倍野	〃
	(15)	難波	〃
市バス	(75)	船町	靱南通

太 融 寺 (宿舎)

	系統番号	行 先	下車場所
市バス	(32)	守口	太融寺
	(ト32)	守口車庫前	〃
	(33)	北清水	〃
	(36)	鶴見町	〃
	(ト36)	安田	〃
	(37)	江口橋	〃

徒歩では大阪駅東口より 10 分，太融寺より大会会場へは，徒歩で市電梅ケ枝町 (約 5 分) へでて出入橋まで市電を利用するのが便利

会 場 案 内 図

市 電
地 下 鉄
徒 歩
城 東 線
東 海 道 線
大 阪 駅
阪急電車
駅前
阪神電車
太融寺(宿舎)
市大理学部
扇橋
南森町
出入橋
桜橋
梅田新道
梅ヶ枝町
堂 島 川
中 之 島
中央公会堂
阪大(会場)
医
理
朝日新聞
土 佐 堀 川
肥後橋
淀屋橋
北浜二丁目
天神橋
N
御堂筋
堺筋
自然科学博物館
ウツボ公園前
シナノ橋
本町四丁目
本町二丁目

Bot. Mag. Tokyo, **73**: 447—452 (December 25 1960)

Developmental Mechanics of Fucaceous Algae XV. Effects of Ultracentrifuging at Later Stages upon the Development of *Coccophora* Eggs*

by Singo NAKAZAWA**

Received May 2, 1960

In eggs of *Fucus furcatus*[1]) and of *Crystoseira barbata*[2]), the polarity axis is determined by stratification of the intracellular materials brought about by means of centrifuging, so that the rhizoid tends to be formed at the centrifugal end when the centrifuged egg is cultured in normal sea water. But, centrifuging is not effective in some other fucoids, *Fucus serratus*[3]), *Coccophora Langsdorfii*[4]), *Sargassum confusum*[5]), and *S. tortile*[6]) in spite of the fact that almost the same stratification can be induced by centrifugal force. In *Fucus furcatus*, the duration of time in which the centrifugal force is effective for polarity determination is restricted to until 12 hours after fertilization. So that, after this the centrifugation is powerless for the determination[1]). In *Coccophora Langsdorfii*, centrifuging experiments were carried out formerly only during the time before to 40 minutes after fertilization[4]). Therefore, it is questioned whether or not centrifuging is also invalid for polarity determination if the egg is centrifuged at a later stage. *Coccophora* eggs are spherical or a little elongated at or just after fertilization. Regardless of their original form, they are more or less elongated by their own morphogenetic movement to become ovate forms pointed towards an end. After this transformation, their polarity axis is determined, and later the rhizoids are formed at the pointed end. The writer here made an experiment to centrifuge egg strongly after the transformation stage, and inspected their later development.

Material and Method

The experiments were carried out in April 1960, at the Marine Biological Station of Asamushi, Aomori Prefecture, Japan. The material, *Coccophora Langsdorfii*, was collected from near the Station, and was cultured in glass vessels with filtered sea water. After liberation, eggs were artificially fertilized, and were strongly centrifuged at stages before and 1, 10, 15, and 20 hours after fertilization. After the centrifugation, the stratification was inspected by use of a microscope, and then they were cultured in filtered sea water contained in Petri dishes. Conveniently, the stratification of plastids is retained without being redistributed even until the stage of young embryos, so that the original direction towards which the egg was centrifuged can be distinguished with ease in later stages. Upon the centrifugation, the material was placed in glass tubes of 4 mm. in diameter and 12 mm. in length, the tubes containing the material were set in an air-turbine centrifuge of 15 mm. in radius, which was turned for five minutes at 25000 times gravity. In *Coccophora*, the development proceeds not always uniformly, but more or less differs according to the individual egg. Therefore, though centrifuged at the same time after fertilization, some eggs undergo stratification at the stage of the primary morphogenetic movement, but other

* Supported by a grant from the Saito Gratitude Foundation, Sendai.

** Biology Department, Yamagata University, Yamagata, Japan.

eggs at two-cell stage or another. Conveniently, however, as was remarked before, the position of plastid layer, which is still retained, indicates the stage at which the egg was centrifuged (Fig. 1 H, I, J, K, L, M).

Observations

The unfertilized egg is spherical or a little elongated containing one nucleus in the central region surrounded by a number of plastids (Fig. 1A). By means of centrifuging, the egg protoplasm is stratified into five layers (Fig. 1B). At the centripetal end, there come dark yellow substances which are presumed to be composed of oil or fat, stainable pink with Sudan III. This layer is liable to be thrown out of the egg cell into the space surrounding the latter when the egg is centrifuged before fertilization. Next to this, a transparent layer is stratified, then a dark brown layer composed of plastids and the nucleus, a colorless clear zone, and finally a layer composed of fine grey particles extending to the centrifugal end. The same stratification appears regardless of the form of the egg. Later than one hour after fertilization, the stratification takes place in a little different pattern. That is, the oil drops, which also appear at the centripetal end, are never thrown out of the egg (Fig. 1C), indicating that a certain change took place in the cell membrane after fertilization. Presumably, deposit of cellulose seems to be such a kind of change. One hour after fertilization most of the eggs still retain their original form as the time is earlier than the occurrence of the transformation. In the culture of these stratified eggs, it is known that the formation of the primary rhizoids takes place regardless of the site of stratification as was reported before[4,5,6].

10 hours after fertilization, the egg undergoes the peculiar transformation into ovate forms pointed towards one end (Fig. 1D). Centrifuged at this stage, the stratification appears in various directions according to the orientation in which the egg is placed by chance upon the centrifugation (Fig. 1 E, F, G). When these eggs were cultured, it was revealed that rhizoid differentiation was affected by centrifuging. In normal development, the presumptive part where the rhizoid pole is determined appears at the pointed end of the transformed egg[6]. This part is called the

Table 1. Number of eggs centrifuged apically, laterally, and basally at the transformation stage, and ratio of their rhizoid formation.

	Apically centrifuged	Laterally centrifuged	Basally centrifuged	Control (without centrifuged)
Number observed	62	68	70	100
Rhizoid formation %	91	90	2	94

basal end. The opposite part, the apical end, is the site where the embryo apex is differentiated later. If the egg is centrifuged basally, the oil cap appears at the apical end of the egg (Fig. 1E), so that this is called "basal centrifugation". But if it is centrifuged apically, the oil cap appears at the basal end (Fig. 1G), i.e. "apical centrifugation". In the same sense, "lateral" (Fig. 1F) and "oblique" centrifugations

Fig. 1. A, normal egg before fertilization; B, the same centrifuged, oil drops are arrowed; C, centrifuged after fertilization; D, transformation stage of a normal egg; E, the same centrifuged basally; F, centrifuged laterally; G, centrifuged apically. H, young embryo forming rhizoids resulting from an apical centrifuging; I, young embryo failing in rhizoid formation resulting from a basal centrifuging; J, young embryo forming rhizoids resulting from a lateral centrifuging. K, basal centrifuging at two-cell stage; L, embryo resulting from the same. M, embryo resulting from an apical centrifuging at two cell stage N, redistribution of the nucleus (arrowed) after stratification; O, a normal embryo.

are possible. In the culture of these centrifuged eggs, it was revealed that (1) the eggs apically or laterally centrifuged could form rhizoids normally at the presumptive rhizoid end (Fig. 1 H, J). However, (2) the eggs centrifuged basally could not form a rhizoid (Fig. 1 I, Tab. 1). In apical centrifugation, the apical half wanting plastids sometimes fails in cell division resulting in a half embryo reported in a previous paper[7]. Though a rhizoid is not formed in the egg centrifuged basally, irregular cell divisions take place. In this case, most of the plastids and the oily materials are retained without being redistributed. However, the nucleus is promptly replaced to the center, and undergoes division (Fig. 1 N). In the normal development, the primary segmentation wall is formed perpendicular to the long axis of the egg. If the egg is centrifuged at two-cell stage or later, the rhizoids are formed normally (Fig. 1 K, L, M). Thus, the ultracentrifuging experiments revealed the relation between the stages at which eggs were centrifuged basally and their later potency of rhizoid differentiation as shown in Fig. 2. That is, when eggs are centrifuged before fertilization, 70 per cent of them can form rhizoids at the presumptive rhizoid end. As the distinction

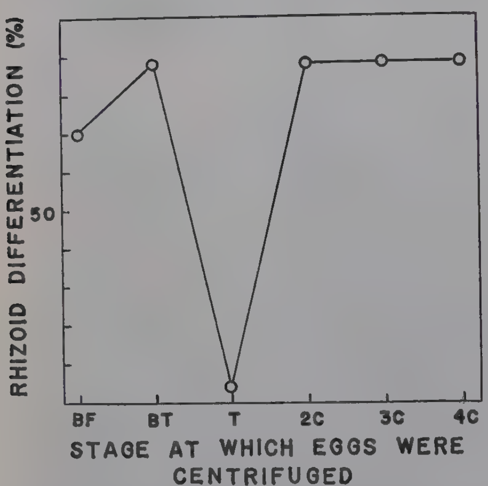


Fig. 2. Rhizoid differentiation after being centrifuged basally at various developmental stages.

of the base and the apex is not always easy in spherical eggs before actual transformation, the observation was restricted only to the eggs whose form was originally ovate pressed by the mucilaginous coat surrounding them. For this purposes, these eggs were cultured isolated from other ones. The percentage of forming rhizoids at the presumptive end rises when the eggs are centrifuged after fertilization but before the transformation to 88 per cent. But when they are centrifuged at transformation stage, it sinks down to only two to four per cent, then rises again when centrifuged at two-cell stage or later.

When the embryo resulting from the centrifuged egg is immersed in sea water where 0.01 per cent brilliant green is dissolved, the cytoplasm is stained vitally. At this time the staining appears from the basal pole or the rhizoids, which is also the case in the abnormal embryo without being centrifuged[8]. In addition, it is noteworthy that even in the normal embryo failing in rhizoid formation resulting from the basal centrifuging (Fig. 1 I), the vital staining also tends to appear from the presumptive rhizoid pole, i.e. the basal end.

Discussion

The most notable facts observed in the above experiments are that rhizoid formation is effected by direction of the stratification when the egg is ultracentrifuged after morphogenetic transformation. That is, when the plastid layer was stratified laterally or in basal regions, the rhizoids were formed normally. However, the rhizoids are not differentiated when the plastid layer was stratified in apical regions. This relation is illustrated in Figure 3, and it will be explained as follows. Judging from the facts above, it seems that the localization of intracellular materials takes part in the differentiation of rhizoids. Though the main material for this is not clear at present, it is certain that it is one of the substances stratified by ultracentrifuging. But it is also clear that the main factor is not concerned with the nucleus. Because the nucleus is promptly replaced to the central region before occurrence of the cleavage in whatever direction it was stratified (Fig. 1 N). Therefore, it must be cytoplasmic materials that take part in the rhizoid formation. However, it is not true that the differentiation of rhizoids is conditioned only by intracellular materials. If it were so, the rhizoid would be formed on any side of the ovate egg corresponding to the direction of the centrifugation. But the fact is different. That is, the rhizoid formation is inhibited when the plastid layer is stratified to the apical region, i.e. far from the presumptive rhizoid pole where the rhizoids were to be formed in normal development. Therefore, it seems that rhizoid formation is also dependent upon the cortical cytoplasm which is not moved by centrifugal force. That is to say, there must be two main factors controlling rhizoid formation. One is the polar differentiation of the cortex of the egg cell which determines the site of the differentiation. The other is a certain intracellular cytoplasmic materials movable by centrifugal force whose localization is important for actual formation of rhizoid. It seems that the rhizoids cannot be formed if the intracellular materials are distributed too far from the presumptive rhizoid pole. In normal cases, the materials are almost uniformly distributed in the cell, so that rhizoid differentiation takes place at the presumptive part. But, if the materials are taken off from the presumptive rhizoid pole by means of centrifuging, the rhizoid formation fails. It is known that the presumptive rhizoid pole is determined at or sometimes before fertilization[6,9]. It is also reported that in *Coccophora* the rhizoids are formed but their site of appearance is not determined by centrifuging when the egg is centrifuged before or just after fertilization[5,9]. At this time, it is considered that the powerlessness of centrifuging in determination is attributed to prompt redistribution of the materials necessary for rhizoid formation to their original location corresponding to the presumptive rhizoid pole. When the egg is centrifuged at two-cell stage, it cannot affect the rhizoid formation even though the plastid layer is stratified apically in each cell. Therefore the plastids do not seem to be the main factor for rhizoid formation.

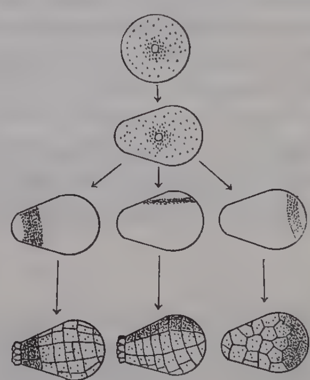


Fig. 3. Developmental fates of *Coccophora* eggs centrifuged in various directions at the transformation stage.

Summary

Eggs of *Coccophora Langsdorfii* were cultured after being ultracentrifuged at

25000 time gravity for five minutes at the time before and 1, 10, 15, and 20 hours after fertilization. As a result, the following was revealed.

(1) When the plastid layer is stratified in apical regions of the egg after transformed to ovate forms, the rhizoid cannot be formed. However, when the same layer is stratified laterally or in basal regions, normal rhizoids are differentiated at the basal, i.e. the pointed end.

(2) Therefore, distribution of the intracellular materials is a factor in the actual formation of rhizoids. But, as the site of the nucleus stratified is not concerned with this, distribution of certain components of the cytoplasm seems to take part in the rhizoid differentiation. It is, however, not the only factor. It seems that (a) the site of the rhizoid differentiation is determined by the cortical layer of the cytoplasm immovable by centrifuging and (b) its actual formation is performed by a certain kind of cytoplasmic elements movable by centrifuging.

(3) When the egg is centrifuged at two-cell stage or later, the rhizoid formation is not influenced by it.

The writer's thanks are due to Prof. Arika Kimura and Prof. Isao Motomura, and to all members of the Marine Biological Station of Asamushi for their kind cooperation in the present study.

References

1) Whitaker, D. M., Growth Supplement **75**: (1940). 2) Knapp, E., Planta **14**: 731 (1931). 3) Beams, H. W., J. Mar. Biol. Assn. Uni Kin. **21**: 571 (1937). 4) Nakazawa, S., Sci. Rep. Tohoku Univ. 4th Ser. **19**: 73 (1951). 5) ——, ibid. **18**: 424 (1950). 6) ——, Naturwiss. **46**: 333 (1959). 7) ——, Bot. Mag. Tokyo **68**: 232 (1955). 8) ——, Sci. Rep. Tohoku Univ. 4th Ser. **20**: 89 (1953). 9) ——, ibid. **23**: 119 (1957).

摘　　要

中沢信午: フークス科藻類の発生力学 XV. スギモク卵のよりおそい発生段階における超遠心の影響

スギモク (*Coccophora Langsdorfii*) 卵が受精後 1, 10, 15, および 20 時間を経過した段階において重力の 25000 倍で 5 分間超遠心してから培養した結果，つぎのことがしられた．

(1) 卵が造形運動によって卵形に変化した段階で超遠心し，プラスチッドが頂部予定域にあつめられたものは仮根形成能を失なう．しかしプラスチッドが基部予定域または側面にあつめられた場合は仮根形成能は正常の場合とひとしく保存され，仮根は卵のとがった部分に形成される．

(2) したがって，仮根が形成されるためには卵内容の分布がひとつの因子になっていることがわかる．しかし核の位置は仮根形式には関係がない．したがって細胞質のある要素の分布が仮根分化に役割を演じていることになる．しかしそれが唯一の因子ではない．おそらく (a) 仮根形成の位置については遠心力でうごかない皮部細胞質がこれを決定し，(b) 仮根の実際の形成には遠心力でうごかされうる細胞質のある要素がはたらいていると考えられる．

(3) 2 細胞期またはより以後で超遠心をおこなっても仮根形式はまったく影響をうけない． (山形大学生物学教室)

Bot. Mag. Tokyo **73**: 453—457 (December 25, 1960)

On the Germination of Pollen Grains of *Brassica napus* L.

by Atsushi KUBO*

Received January 20, 1960

The writer is planning to achieve the comparative study on conditions and rate of germination of pollen grains. Pollen grain of *Brassica napus* L. was chosen as the material. At present there are neither suitable culture conditions nor good germination found as to the pollen grains of any *Brassica* plant. Sasaki[1]) reported pollen grains of *Brassica campestris* L. could germinate on sucrose-agar medium, but 12.2% germination in the best was found only on the 1.0% agar medium containing 30% sucrose. Shisa[2]) said that 54.6% of pollen grains of a *Brassica* plant (Suigukina) could germinate under special conditions such as pH 7.6. Thus pollen grains of *Brassica* could not germinate easily on culture medium. The present writer found pollen grains of Compositae[3]), *Triticum vulgare*[4]) and *Zea Mays*[5]) could germinate more favourably on the thin layer of gelatin medium. In this paper the writer will report the results obtained by experiments with pollen grains of *B. napus* cultured on gelatin media.

Material and Method

Culture experiments of the pollen grains of *Brassica napus* L. were done during April 6—9. The flowers of the plants in the field were used. Yellow coloured-gelatin** (yellow-gelatin), gray coloured-gelatin** (gray-gelatin) both made by Yasu Chemical Industrial Co., Shiga Prefec. in Japan, and colourless-transparent-gelatin** (Merck-gelatin) made by E. Merck in Germany, were used. These lean or sweetened media were warmed and materials in sol state were pasted thinly on slide glass by the same method as the former experiments[3-6]). When the medium was solidified by cooling in the air, the pollen grains were seeded with a small blush. The preparation was put into petri-dish of 18 mm. in depth and 90 mm. in diameter in which the humidity was kept with a piece of wet filter paper. In five fields, each containing about 100 grains, the number of germinated grains was counted, then their percentages were calculated.

Experimental Results

Exp. 1 Favourable culture conditions

Lean and sweetened media containing various gelatin were made to optional 20μ thick layers, then the pollen grains were seeded on them. They were kept in the moist chamber (100% R.H.) in which the humidity was kept with wet filter paper adhered to all of the inside walls of a petri-dish. Germination was seen on 30—70% yellow-gelatin at the room temperature and the best germination ratio was 24.1% on 10% sucrose–60% gelatin. On gray-gelatin, in the range of 20—60% germination was seen and the best germination was 7.8% on 10% sucrose–50% gelatin. No germination was seen on Merck-gelatin. As the medium of 10% sucrose–60% yellow-gelatin was the best for the germination of the pollen grains of *B. napus*, the same medium will be used in the following experiments.

---

* Crop Science Laboratory, Faculty of Agriculture, University of Kyushu, Fukuoka, Japan.

** The gelatins are all manufactured from the same origin namely cow, pig and whale. The colour is diverse, corresponding to their quality.

a) Thickness of medium

Various thick media of 10% sucrose-60% gelatin were prepared and the thickness of them were ascertained by the way as shown in Figure 1. Pollen grains are seeded

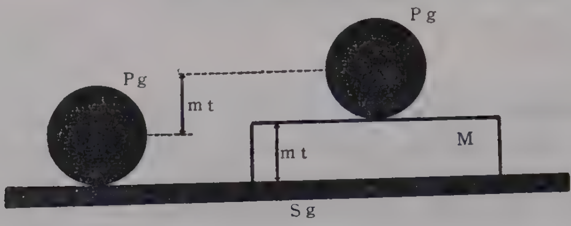


Fig. 1. The measuring way of medium thickness.
Pg: Pollen grain M: Medium
Sg: Slide glass mt: Medium thickness

on each of the two positions of the slide glass one position of which is pasted with the medium and the other is not. The microscope was focussed to the pollen grain on the former position, and then to that on the latter one. The difference between both pollen grains at the two positions was measured by reading the revoluting paces of the microscrew at focussing; this difference is equal to the thickness of medium layer. Pollen grains were seeded on media of various thickness and those preparations were kept in 100% R.H. at the room temperature. The results was shown in Table 1. The best germination ratio was 75.3% on the 40μ thick medium. In this

Table 1. Germination and plasmoptysis of pollen grain in accordance with the thickness of medium of 10% sucrose-60% gelatin at 17°.

Thickness of medium (μ)	10	15	20	25	30	35	40	45	50	60	70	80
Percentage of germination	1.2	4.8	22.6	38.3	46.7	68.5	**75.3**	48.9	42.5	11.8	0.9	0
Percentage of bursting	21.3	24.3	58.5	42.0	28.3	17.7	**14.5**	7.0	0	0	0	0

case 14.5% plasmoptysed pollen grains were seen. They increased on the media thinner than 40μ and 58.5% plasmoptysed pollen grains were seen on the 20μ thick medium. On the other hand on the medium thicker than 40μ, plasmoptysed pollen grains decreased and at last on the thick medium over 50μ, they were none.

b) Humidity in the moist chamber

A round wet filter paper of 11 cm. in diameter and then 1/2, 1/4, 1/8 and 1/16 pieces were put into petri-dishes. They contain 1 m*l*. water. Thus, every chamber of various moisture was prepared. Each of the 40μ thick media of 10% sucrose-60% gelatin where pollen grains were seeded was put into each of those moist chambers at the room temperature (17°). The result was shown in Table 2. The best germination ratio was 98.7% in the humidity by 1/4 piece of a wet filter paper in width and in that case plasmoptysed pollen grain was none. The wider the filter paper, the more plasmoptysed pollen grain increased.

Exp. 2. Germination in the improved culture condition

40μ thick media of various concentrations of lean and sucrose gelatin were prepared and the pollen grains were seeded on them. The preparations were put into

Table 2. Germination and plasmoptysis of pollen grain in accordance with degree of the width of round wet filter paper (r=55 mm.) on the 40μ thick medium of 10% sucrose–60% gelatin at 17°.

Width of round filter paper (r=55 mm.)	1	1/2	1/4	1/8	1/16
Percentage of germination	75.3	95.1	**98.7**	94.1	91.3
Percentage of bursting	14.5	4.5	0	0	0

the moist chambers where the humidity was kept by 1/4 piece of the wet filter paper at the room temperature. Germination ratio and tube length are shown in Table 3.

Table 3. Germination ratio and the tube elongation on the sucrose-gelatin medium in the moist chamber with 1/4 wet filter paper at 17°.

Concentration of sucrose (%)	Concentration of gelatin (%) 5	10	20	30	40	50	60	70	80	90
	Germination ratio (%)									
0	0	0	42.9	**94.6**	23.5	14.5	**85.7**	28.0	70.5	48.2
10	2.6	0	18.6	56.2	18.1	48.0	**98.5**	0	0	0
20	0.8	8.0	27.9	70.6	27.5	32.0	95.0	0	0	0
30	0	2.5	12.6	17.3	20.5	28.6	39.1	4.5	0	0
	Tube length (μ)									
0	0	0	630	**2240**	336	420	840	560	168	48
10	126	0	350	630	980	**1960**	**1400**	0	0	0
20	28	84	224	980	630	1680	616	0	0	0
30	0	28	490	168	126	560	210	48	0	0

Good germination ratios were seen specially at two concentrations of 30% and 60% in lean gelatin medium. They were 94.6% and 85.7%. And the best germination ratio was 98.5% on 10% sucrose–60% gelatin medium in sucrose-gelatin medium. On the other hand the longest tube was 2240μ on 30% gelatin medium. Next the long tube was 1960μ on 10% sucrose–50% gelatin. But, the tube length was 1400μ on 10% sucrose–60% gelatin which showed the highest germination ratio.

Exp. 3. Shapes of germinating pollen grains

The set as shown in Figure 2 was used, in order to observe the varied shapes of germinating pollen grains. That is, 10% sucrose–60% gelatin which showed the best result was pasted in the thickness of 40μ on a slide glass and the frame of a wet cardboard was set on it, then the frame was covered with the cover-glass. Thus, a moist chamber was prepared. And the pollen grain was observed under the microscope as it was in the chamber. Variations of its length and width were as in Table 4. In 3 minutes after the pollen grain was placed on the medium, its length shortened and its width extended quickly. In 15 minutes they changed very slowly, after that the change was not seen at all. After 34 minutes pollen tube began to develop.

Table 4. Variation of the size of pollen grain.

Time (min.)	0	3	9	15	27	34		
Length (μ)	49.0	44.8	42.0	40.6	40.6	40.6	Germination ratio	92.8%
Width (μ)	26.6	28.0	29.4	32.2	32.2	32.2	Bursting	0 %

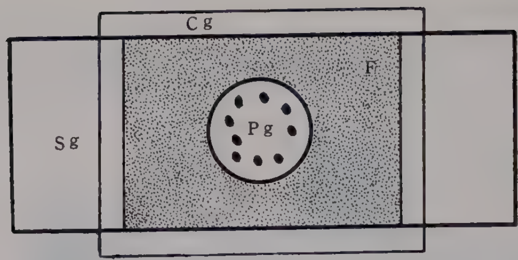


Fig. 2. The observation method of germinating pollen grain.
Pg: Pollen grains F: Frame
Sg: Slide glass Cg: Cover glass

Discussion

When the pollen grain of *B. napus* germinates, it changes its form and swells quickly at the first stage, but later such change is scarcely seen. Such phenomenon has already been observed also in the pollen grain of *Triticum vulgare*[4]. And such form of water absorption seems to be just achieved by the mechanism in the thin layer of hypertonic gelatin medium as follows. The more thinly the medium is pasted on a slide glass, the more quickly gel change of medium progresses. The pollen grain seeded on such very thin layer of gel medium can not contact well to it. Therefore, there is no water relation between medium and pollen grain. But the moisture in the air is sucked gradually into it by the action of hydrature equilibrium[6] and deliquescence in medium. Consequently free water become abundant near the surface of medium. In this case the pollen grain should suck the water quickly to be swollen. On the other hand the water penetrates into the deeper part of the medium, then the medium becomes sticky or swells. So, the layer increases even its thickness[7]. For that reason, the pollen grain is close enough to the medium. When the pollen grains of some plants[2,4,8] could not suck the water from the thick layer of hypertonic gelatin medium as in this case, the water absorption is controlled strongly by the swollen medium too. Water is sucked enough at the first stage and only little at the later stage. Hardly germinable pollen grains generally can not regulate water absorption by their own ability[9], so the swelling form of pollen grain to germinate is owed to the feature of water supply from medium. Tokugawa[10] succeeded in germinating the pollen grain of some plant of Compositae on the medium when sucrose solution was given slightly on the slide glass. Walderdorff[11] dried 10% gelatin solution and allowed the pollen grain of *Oenothera* germinate very well. By the writer[9] it is verified that the pollen grain of *Cosmos* which could not germinate on the thick sucrose-agar medium over 1 mm. could germinate on the 10μ thick medium. Therefore there is nothing but to be thought that water supply in the thin layer of medium benefited germination. Of course, there are questions in the explanation of Kuhn[12] that the thin layer of agar medium amasses the special substance which stimulates the germination. But though the thin layer of medium enables them to germinate the above mentioned pollen grains, the results were not satisfactory. Such results were seen also in the other gelatins and the germination was not always good. It will be owed to that the form of water supply of medium is unfavorable to the pollen grain. The form of the water supply was differed by the quality or thickness of medium. And the optimum water absorption is given only by some gelatin (yellow one) in various ones. Such difference of the germination may be due to components contained in gelatin as amino acids. Sawada[13] had already confirmed that some amino acids stimu-

lated the germination action of pollen grain very well. However, the writer can not pass over that amino acid or other substances in medium may vary the condition of the water supply from it or water suction by pollen grain.

Summary

1) The pollen grain of *Brassica napus* germinated best on the 40μ thick medium of 60% yellow gelatin contained 10% sucrose, but the pollen grain plasmoptysed very much in the 100% relative humidity. The thicker the medium, the more plasmoptysed pollen grain decreased.

2) Even on the medium of optimum concentration the pollen grain plasmoptysed in the 100% R. H. of the moist chamber, but in lower humidity no pollen grain plasmoptysed and the highest rate of 98.5% germination was obtained in the optimum humidity.

3) The pollen tube was the longest, 2240μ, on the 30% lean gelatin medium. But the tube length was 1400μ on the 10% sucrose-60% gelatin which permitted the highest germination. The tube length did not always go together with germination ratio.

4) The water economy should exist between the pollen grain and the medium, as follows. When the thin layer of gelatin medium absorbed air moisture by the action of deliquescense and hydrature equilibrium, the moisture stay at its surface, the pollen grain sucking the water and swelling quickly. Then, the water gradually penetrates into the deeper part of the medium accompanying the decrease of excess moisture from the medium surface. Thus the water absorption of the pollen grain is controlled properly for germination.

References

1) Sasaki, T., Sci. Agr. Soc. Tokyo **207**: 921 (1919). 2) Shisa, M., J. Hort. Assoc. **5**: 105 (1934). 3) Kubo, A., J. Sci. Hiroshima Univ. **7**: 23 (1955). 4) ——, ibid. **7**: 103 (1956). 5) ——, Bot. Mag. Tokyo **71**: 282 (1958). 6) Walter, H., Jena (1931). 7) Kubo, A., Unpublished (1960). 8) ——, Jap. J. Bot. **16**: 15 (1955). 9) ——, J. Sci. Hiroshima Univ. **6**: 234 (1954). 10) Tokugawa, Y., Bot. Mag. Tokyo **28**: 494 (1914). 11) Walderdorff, M. v., Bot. Arch. **6**: 84 (1924). 12) Kuhn, F., Planta **27**: 304 (1937). 13) Sawada, Y., Bot. Mag. Tokyo **71**: 218 (1958).

摘　　要

久保　淳: *Brassica napus* L. の花粉の発芽について

B. napus L. の花粉は 40μ の厚さの 10% 蔗糖 60% 黄色ゼラチン発芽床で最もよく発芽した．しかし湿度 100% では吐出花粉が非常に多かった．発芽床の厚さがうすくなるほど吐出花粉は多くなり，厚くなるほど少なくなった．発芽に最適な濃度の発芽床においてさえ空中の湿度が高すぎると花粉は吐出する．しかし湿室内の湿した沪紙の広さをせばめ湿度を下げると吐出花粉は減少した．そして適度の湿度では最高 98.7% の発芽成績が示された．花粉管は蔗糖を含まない 30% ゼラチン発芽床上で 2240μ の伸長度を示した．しかし最高の発芽成績を示した 10% 蔗糖−60% ゼラチン発芽床では 1400μ であった．すなわち花粉管の伸長度は必ずしも発芽成績とは伴わない．

花粉と発芽床との間には次のごとき水分経済が存在すると思われる．すなわち薄層ゼラチン発芽床がその潮解性と水分均衡の両作用によって空中から湿気をとった時水分は最初はその表層附近にとどまる．花粉はこの時急速に吸水し，かつ膨潤する．他方水分は次第に発芽床の内部に浸入しつづけるために発芽床は漸次膨潤するし，したがって発芽床表層附近の水分も少なくなる．花粉はこの時吸水を抑圧される．

(九州大学農学部作物学教室)

Bot. Mag. Tokyo, **73**: 458—467 (December 25, 1960)

Photosynthesis Pattern of Natural Phytoplankton Relating to Light Intensity*

by Shun-ei ICHIMURA**

Received May 4, 1960

Since the dry matter production of plants depends on their photosynthesis, the characteristics of the latter in natural phytoplankton have gradually become manifest with the progress in the study of the primary production in waters.

Among the various features of photosynthesis, the information on the characteristics of the light-photosynthesis relation seems to give us the most basic knowledge for the analyses of the primary production in phytoplankton community. This precise information especially makes the indirect approach to the primary production such as the chlorophyll and tank methods as accurate as possible, whereby the primary production is assessed with combining the photosynthesis-light curve and the illumination in water.

As has been well known by plant ecologists, in the leaves of the terrestrial plants, two patterns of photosynthesis—sun and shade leaf patterns—can be classified with the features in the photosynthetic response to varying light intensities. Concerning the said photosynthesis patterns, however, it has not yet been investigated adequately in natural phytoplankton, though contributive studies were reported recently by several investigators as Talling[1]), Rodhe *et al.*[2]), Steemann Nielsen[3]), and Ryther and Menzel[4]). The present study has pursued the light-dependent photosynthesis patterns in natural phytoplankton and also the process of the differentiation of the patterns.

Methods

The sample waters used in this study were taken from various depths of a lake by hand pump. The photosynthesis was measured by the ^{14}C method in oligotrophic samples and by the Winkler method in eutrophic samples as employed in a previous study[5]). The sample waters filled in 100 m*l*. glass bottles were illuminated in a water bath with a 500W flood lamp. The light intensities falling on the bottles were regulated with varying distances of the light source. After 2–3 hr. illumination, the photosynthesis in clear bottles and the respiration in dark ones were determined. The standing crop of phytoplankton in the sample water was measured by means of chlorophyll amount determined by the pigment extraction method. The characteristic of the shape of photosynthesis-light curve was indicated by quantity I_k, an equivalent to the light intensity where the extrapolations of the initial linear region in the photosynthesis-light curve and of the light saturation region intersect. This quantity was first proposed by Talling (1957, p. 36)[1]) as an indicator of the characteristic of photosynthesis-light curve. Steemann Nielsen[3]) has also employed I_k as a useful indicator of the photosynthetic characteristic of phytoplankton population. The value of I_k is about 6000 lux in the typical sun form and 1500 lux in the shade form of natural phytoplankton in some Japanese lakes.

* This work was supported by the Grant in Aid of Scientific Research of the Ministry of Education.

** Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan.

Seasonal change in photosynthetic activity of natural phytoplankton

Fig. 1 shows the seasonal change in photosynthetic activity of phytoplankton taken from the depths of 0, 2, 4, 6 and 8 m. in eutrophic lake Fukami*. Each value in the

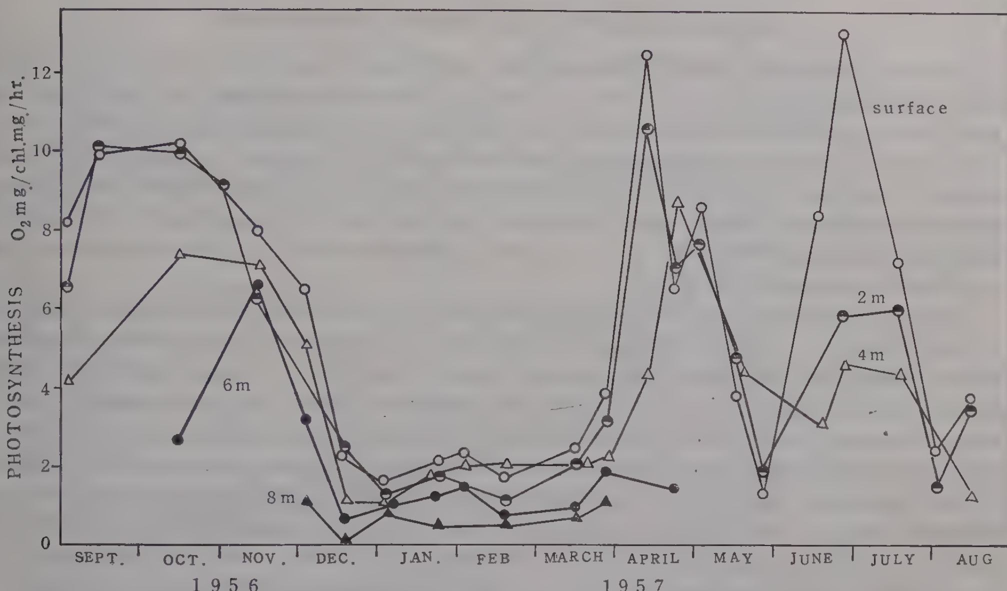


Fig. 1. Seasonal change in photosynthetic activity of natural phytoplankton in lake Fukami (Sept. 1956–Aug. 1957).

figure represents the gross photosynthesis rate obtained at the light saturation point and the *in situ* temperature. The intensive rate was found in spring, midsummer and autumn, and the lowest one in late summer and winter. These features of the seasonal change resembled fairly well to those obtained by Gessner[6]) and those reported in a previous study[7]), although in the latter the photosynthesis was measured in the surface phytoplankton under field conditions.

In May, a marked decline of photosynthetic rate appeared in the surface layer of lake Fukami, but this may be postulated as an abnormal phenomenon caused by the toxic effect of parathion which entered into the lake from the surrounding paddy fields. According to Sano and Matsui[8]), the photosynthetic activity of phytoplankton was reduced remarkably by the addition of small amount of parathion (1 ppm.) into the culture solution.

The difference in photosynthetic activity was also observed among phytoplankton taken from various depths within a lake, and this could be observed distinctly during the stagnation period in midsummer. At this period, the photosynthetic activity of phytoplankton reached its acme in the surface layer and it successively decreased as the depth increases. During the circulation period in spring and autumn, the activity was nearly the same in all phytoplankters within a photic layer from surface to 4m. depth.

Usually the difference in photosynthetic activity due to the changing depth can be

* Fukami-ike, located in Nagano Prefecture, has an area of 0.02 km^2. and a maximum depth of 9 m.

seen clearly in deep lakes but such difference occurs slightly in the shallow lakes (Ichimura, in press). The above facts may be interpreted on the basis of the photosynthesis pattern characterized by the sun and shade phytoplankton.

Sun and shade forms in natural phytoplankton

In the planktonic algae cultured under controlled illumination, the shade forms can be discriminated in small size and in rich chlorophyll content, but accompanied with low assimilation numbers, from the sun forms. In natural phytoplankton, however, there is no clear morphological discrimination and, moreover, inevitable difficulty exists in precise determination of chlorophyll content in phytoplankton which may hardly be separated from organic matter of various origins suspending in the water. Here physiological distinction of both forms in photosynthetic pattern seems to be the problem remaining to be studied.

The photosynthetic pattern in phytoplankton was examined with the data obtained in lake Fukami, a small, eutrophic and deep lake. It shows a high turbidity with an annual change of the Secchi disk reading from 0.6 to 1.9 m. According to the 1956 and 1957 measurements *in situ* of the photosynthetic rates, the compensation depth ranges between 3—4 m. throughout the year. Dominant species constituting the phytoplankton community were diatoms and green algae, and the taxonomical difference of phytoplankton species could not be observed in vertical distribution as well as in seasonal change. Although the benzol extracts from the samples of various depths indicated brown or bright green color, the absorption spectra were almost similar.

In Fig. 2 are represented some of the photosynthesis-light curves of waters sampled from the depths of 0, 2, 4 and 6 m. with 2-week intervals throughout the year. There, the characteristic photosynthesis-light curves, which correspond to the well-known photosynthesis curves in the sun and shade leaves of terrestrial plants, can be clearly found in natural phytoplankton.

The sun type photosynthesis curve was usually obtained in the surface phytoplankton in the early summer and autumn, and the light saturation point was indicated about 15—20 kilolux and the compensation point 600 lux. On the contrary, the phytoplankton taken from the deeper layer showed mostly the shade type with 5—6 kilolux of the light saturation point and 200 lux of the compensation point. Furthermore, it is noticeable that the photosynthetic rate within the range of low light intensities increases linearly with increasing light intensity and each photosynthetic rate represented per unit amount of chlorophyll fits fairly well to an identical line, although the photosynthesis-light curve in each sample indicates the characteristic pattern according to the difference in depth. Such feature in photosynthesis curve has already been reported by Steemann Nielsen[3]) in marine phytoplankton. This fact seems to be important for the ecological study of the primary production, because under field conditions deeper phytoplankton is generally exposed to underwater illumination, in which every photosynthesis-light curve falls on each other. Hence, the dry matter production estimated by using the photosynthesis curve of the surface sample may not particularly depart from the value obtained by using the photosynthesis curves of the samples from various depths.

In the shallow lakes, marked differentiation of photosynthesis pattern does not appear in vertical but in seasonal. Fig. 3 shows the seasonal change in photosynthesis pattern in the surface phytoplankton taken from lake Kasumigaura, 3 m. in mean depth and 178 km.^{2} in surface area. Dominant species of the phytoplankton communi-

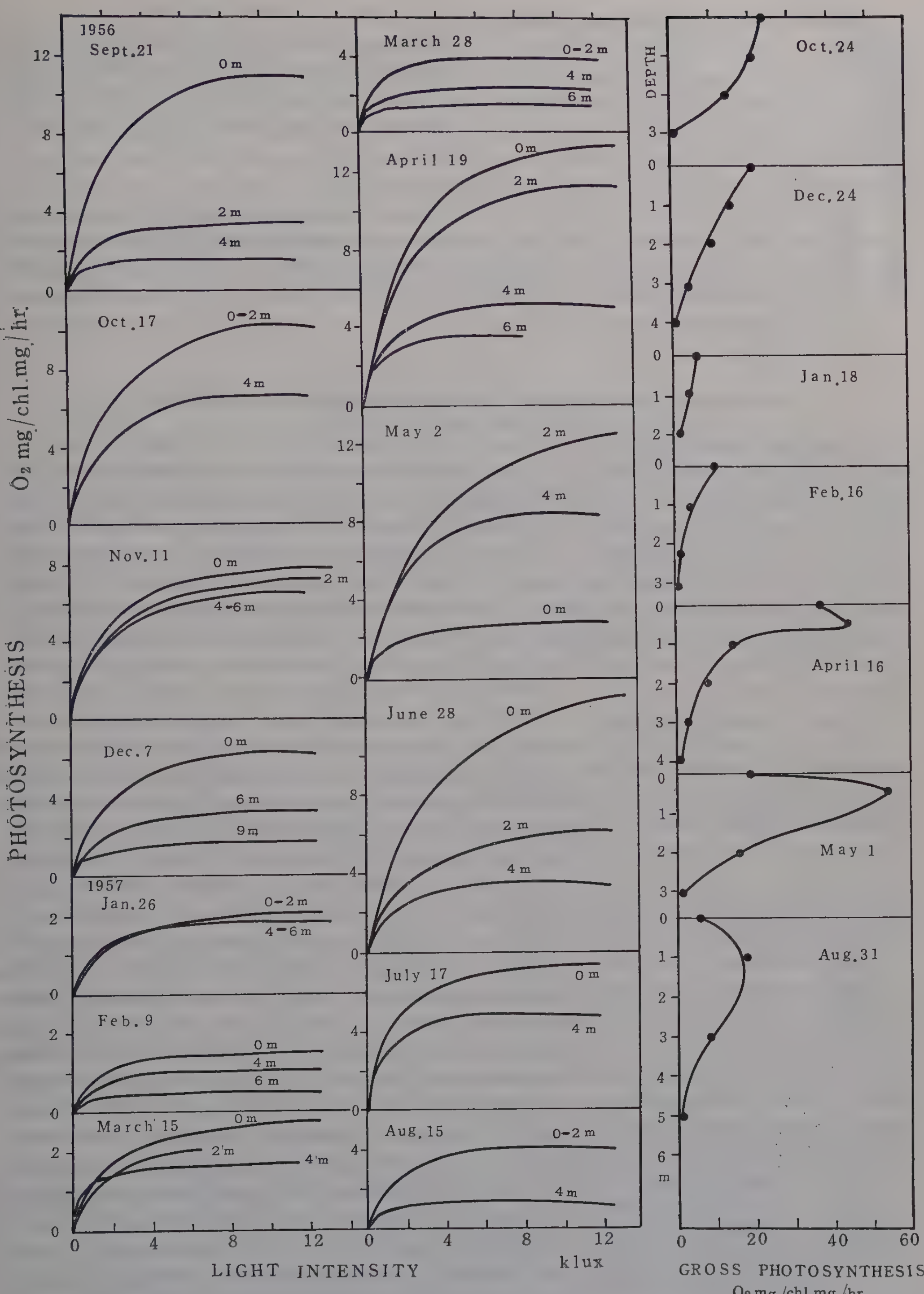
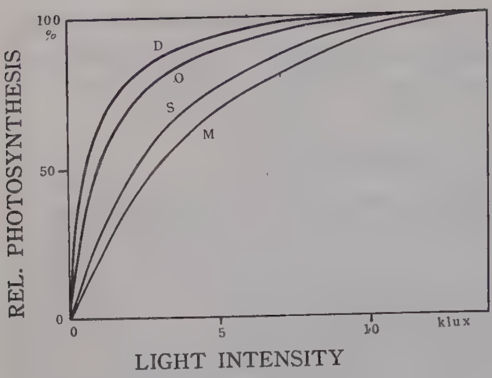


Fig. 2. Photosynthesis-light curves in phytoplankton of varying depths and vertical change in photosynthetic activity in lake Fukami.



ties were *Synedra acus* and *Pediastrum duplex* in winter and spring, while *Microcystis aeruginosa* in autumn. The sun form was found in the early-summer phytoplankton and the shade form in the winter one.

Fig. 3. Seasonal change in photosynthesis pattern in surface water from lake Kasumigaura. M: May 16, S: Sept. 16, O: Oct. 27, D: Dec. 14, 1955.

Taxonomical and regional difference in photosynthesis pattern

Using the various kinds of cultured marine phytoplankton, Ryther[9]) obtained a series of photosynthesis-light curves and concluded that the green algae, diatoms and dinoflagellates could respectively be considered as "sun", "intermediate" and "shade" form. According to the study made by Talling[1]) in Windermere, the fresh water diatom showed the shade type. In a previous paper[10]), the author also suggested that in the natural fresh water phytoplankton, green and blue-green algae act as the sun form and diatoms as the shade form. Verduin[11]) found that the photosynthesis-light curve determined in phytoplankton taken on cloudy days was significantly different from that determined on clear days, the former was the shade type and the latter the sun type.

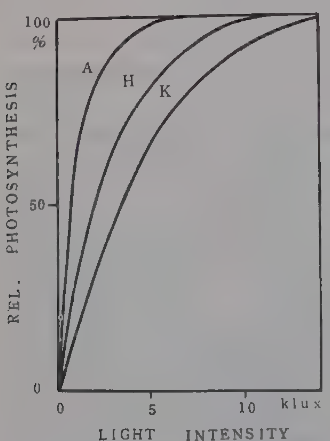


Fig. 4. Regional difference of photosynthesis pattern in surface waters. Measurements were made in May 1956. A: Ashino-ko (oligotrophic), H: Harunako (mesotrophic), K: Kasumigaura (eutrophic).

The photosynthesis-light relation should be discussed on the basis of the data obtained under the optimal conditions for each alga. For that reason the photosynthesis-light curves were compared among the three natural planktonic algae, each of which growing densely in the eutrophic waters. The light saturation of photosynthesis for these curves appears at 18 kilolux in blue-green algae (*Microcystis*), at 15 kilolux in green algae (*Pediastrum*, *Eudorina*), and at 12 kilolux in diatoms (*Synedra*, *Melosira*). All of the photosynthesis-light curves in these planktonic algae indicated on the whole the sun-like type with slight difference. As pointed out by Ryther[3]), the distinction of photosynthesis pattern in different species may not have any ecological meaning on the primary production in natural water, because the above species generally appear together in the same community.

The photosynthetic responsibility of phytoplankton to the varying light intensities also differed regionally through the difference in the environmental factors. By comparing the photosynthesis-light curves obtained in the samples from several lakes which are dissimilar in their character because of regional difference (cf. Fig. 4), some significant differences can be seen among these curves. Each curve was obtained in the sur-

face diatom population at the growing period in May. The phytoplankton communities were floristically nearly the same in three lakes and consisted of *Asterionella gracillima*, *Melosira italica*, *Synedra acus* and *Fragilaria crotonensis*. The curve K indicating the photosynthesis in the eutrophic lake Kasumigaura is a sun-like type and it reaches the light saturated point at ca. 15 kilolux. Whereas the curve A obtained in the sample of the oligotrophic lake Ashinoko presents the typical shade type, of which the light saturation is obtained at about 6.5 kilolux. The sample taken from the mesotrophic lake Haruna indicates the intermediate curve H, light saturation at 12 kilolux. In marine phytoplankton, Steemann Nielsen[3]) observed the obvious regional difference in the photosynthesis pattern, although he could not find any simple correlation between the latitudes and light adaptation of the phytoplankton even at the same time of the year.

Factors affecting differentiation of photosynthesis pattern in phytoplankton

As described above natural phytoplankton can be divided into two forms of sun and shade through the photosynthetic characteristics and this result coincides fairly well with those obtained by Steemann Nielsen[3]), Ryther and Menzel[4]) in marine phytoplankton. However, the factors affecting the differentiation of photosynthesis pattern have not yet been clarified satisfactorily under field conditions. As has been illustrated by many ecologists, the characteristic pattern of photosynthesis-light curve provides the important clue for the analyses of ecological problems in plant communities such as dry matter production, plant succession and shade tolerance, etc., therefore it seems also indispensable for the study of the primary production to clarify the causes determining the photosynthesis pattern.

From the study of the unialgal culture in the laboratory, several factors such as light intensity, temperature and nutrient concentration are surmised as the factors involved in the differentiation of photosynthesis pattern and among them the light is the most essential. The planktonic algae cultured under bright illumination indicate usually the sun type photosynthesis, and the shade type is obtained in the algae which are cultured under weak illumination.

In natural phytoplankton, the photosynthesis pattern changes with decreasing light intensity from the sun type in surface layer phytoplankton to the shade one in deeper phytoplankton. However, it was confirmed by the following experiments that the effect of past history of light condition on algae in photosynthetic character does not remain for a long period after the algae were transferred to the different habitat in light condition. The first experiment was undertaken with the sun form phytoplankton taken from the surface layer of lake Teganuma in June. The sample waters filled in 10 *l*. glass bottles were kept under the variously controlled sunlight in the greenhouse and the change of the photosynthetic character in the samples was pursued. As can be seen in Fig. 5, the conversion could be detected after one day and it was completely established in 10% and 3% of sunlight after 4 days but hardly in 30%.

Another experiment was made in the samples from the depths of 0, 2, 4 and 6 m. in lake Nakanuma in July. According to the difference of sampling depth, the photosynthesis pattern in the initial samples differentiated definitely into the sun type in the surface sample and the shade type in the samples of 2, 4 and 6 m. The sample waters intended for the measurement of photosynthesis were filled in flasks of 2 *l*. and exposed under 8000 lux in a growth chamber at 20°. After 2 days, the photosynthetic

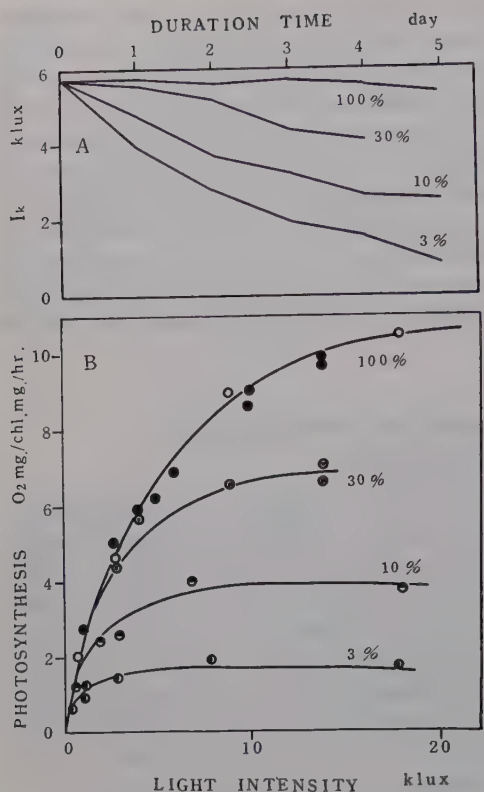


Fig. 5. A) Differentiation of photosynthesis pattern in natural phytoplankton kept under various light intensity. Figures on the lines indicate percentages for direct sunlight. Samples were taken from the surface of lake Teganuma in June 1956. B) Photosynthesis-light curves after 4 days from the start of experiment. ○ denotes initial value.

activity in each sample was measured. As the results, the samples taken from the depths of 2 and 4m. were transformed completely into the sun type. The sample from 6 m. had not any changes in photosynthetic character and remained in its shade type. The chlorophyll amount in the water from 2 m. depth increased from initial value of 0.042 mg./*l* to 0.459 mg./*l* in one month after the sample was kept under natural full sunlight, while that in the water from 6 m. depth increased only its value from 0.014 mg./*l* to 0.018 mg./*l*. Furthermore, the compensation depth determined by means of "*in situ*" measurement was 4 m. From these results, it can be deduced that the shade-like photosynthesis curve obtained in the sample from 6 m. depth may be indicated by the deteriorated phytoplankton.

As another factor referred to the differentiation of photosynthesis pattern, the temperature is normally conceived from the current knowledge on the physiology of planktonic algae. Uusally, algae grown at moderate high temperature under the enriched condition represent the sun type, whereas those cultured at lower temperature show the shade type. Fig. 6 indicates the effect of temperature upon the differentiation of the photosynthesis pattern of natural phytoplankton. The samples from the depths of 0, 2 and 6 m. in lake Fukami in October were incubated 6 hrs. separately in the dark at constant temperature of 22°, 15° (*in situ* temperature) and 7° before the starting of the experiment. In spite of such short incubation, the differentiation of photosynthesis pattern corresponding to the difference in the temperatures was observed. The sample being kept at 22° showed the sun-like type and contrary the shade type in sample at 7°, but the sample from 6 m. depth did not react actively to the difference in temperatures. The experiments described above may suggest that the phytoplankton contained in a layer deeper than the depth of compensation is likely to be deteriorated algae.

The difference in photosynthetic characteristics was also referred to the magnitude of nutrients dissolved in the water. According to Edmondson's experiment[12]) in a small pond, the effect of fertilization on the photosynthetic activity appeared remarkably in 3 days after the fertilization. Also the author[10]) observed that the shade-like photosynthesis pattern in phytoplankton from oligotrophic lake transformed to the sun type only in 2 days after fertilization.

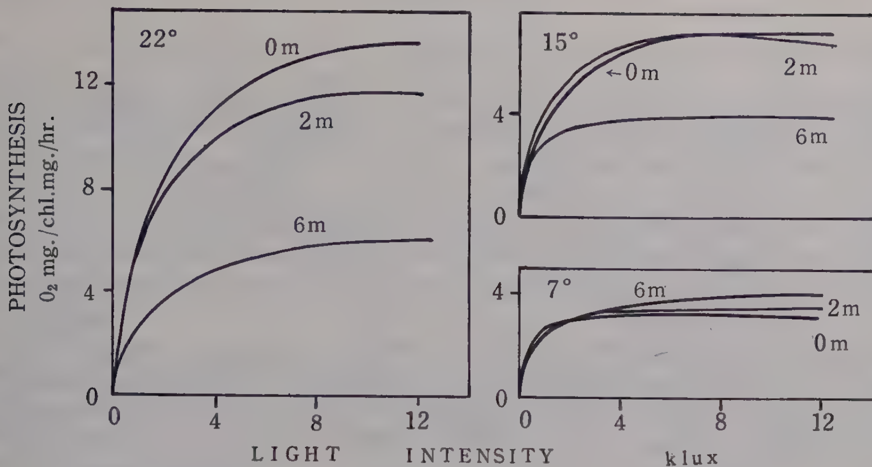


Fig. 6. Effect of temperature on photosynthesis pattern.

During summer season, the nutrients contained in the surface water are usually almost exhausted by phytoplankton even in eutrophic lakes and consequently the photosynthetic activity of phytoplankton decreases. As for these phenomena, an experiment was undertaken with the sample water from lake Teganuma in September 1956. The amount of NH_3-N and NO_3-N in the water was almost nil and PO_4-P could not be detected colorimetrically. After addition of nutrients, the samples were stored for 24 hrs. in a growth chamber at 8000 lux and 20° and then their photosynthetic activities were measured. As seen in Fig. 7, the effect of the fertilization on the photosynthetic activity can be seen clearly and it may also show that the deficiency of nutrient in water transforms the sun type photosynthesis to the shade one.

The serial experiments described above may suggest that the photosynthesis pattern in natural phytoplankton is not the genetic character peculiar to species but determined by the physio-ecological condition of phytoplankton.

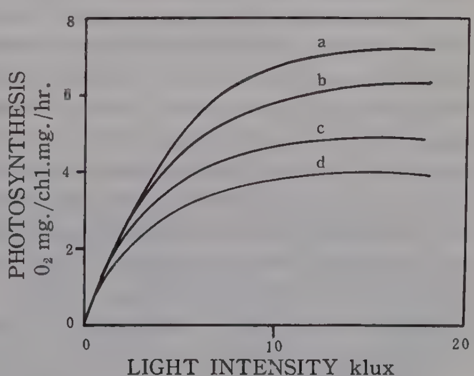


Fig. 7. Effect of fertilization on photosynthesis pattern. a: PO_4-P 0.5mg./l, NO_3-N 0.5mg./l. b: PO_4-P 0.5mg./l. c: NO_3-N 0.5mg./l. d: initial water.

Process of differentiation in photosynthesis pattern of phytoplankton at field

Using I_k, Fig. 8 denotes the seasonal change in the photosynthesis pattern obtained in lake Fukami, and suggests the process of the differentiation of photosynthesis pattern in natural phytoplankton. During the circulation period in autumn, the phytoplankton from the photic layer indicated the nearly sun-like photosynthesis type (I_k=5300 lux). This might be attributed to the vertical mixing of the water mass, through which the surface phytoplankton and subsurface phytoplankton were mixed and they would be exposed moderately to the bright illumination. Moreover, the photic layer was enriched by the upwelling of stored nutrients from the deeper layer and such circumstance may provide an excellent condition for the formation of the sun form phytoplankton.

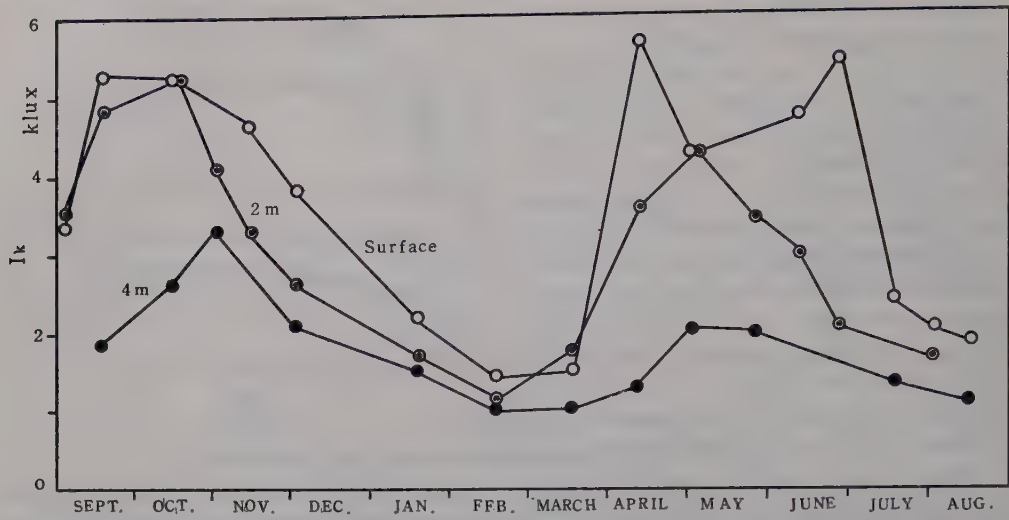


Fig. 8. Seasonal change in photosynthesis pattern denoted by I_k.

These anticipations could be confirmed by the slight graduation in the water temperature and by the increase of nutrient concentration in surface water at the autumn overturn in September and late October, when the circulation of water took place completely. Incidentally, the low turbidity at this period is also surmised as one of the causes relating to the formation of sun form in deeper phytoplankton, because light can penetrate into the deep layer.

In the winter stagnation period, with decrease of the water temperature, the photosynthesis pattern transformed rapidly to the shade type (I_k=1000—2000 lux), while it recovered again to the sun type in the spring surface phytoplankton. Because the spring overturn in lake Fukami was not notable and the mixing of water took place only within the upper layer, a vertical differentiation of photosynthesis pattern was found in the phytoplankton in varied depths, namely I_k was 5500 lux in the sun form surface phytoplankton and 1000—2000 lux in the shade form deeper phytoplankton. This differentiation became more striking in the stagnation period of midsummer. At this period, the agitation of water is slight and the phytoplankton in deeper layer can not shift easily to the upper layer. Consequently, the plankton is kept for a long period under a weak illumination in the deeper layer, though there the water is enriched as a result of the decomposition of the precipitated organic materials.

As indicated in the previous section, the shade-like photosynthesis curve (I_k= 2000 lux) in the late-summer surface phytoplankton undoubtedly must be caused by the deficiency of nutrients, since PO_4–P, NH_3–N and NO_3–N were completely exhausted during this season.

The shade-form phytoplankton in the deeper layer may be referred to the inactive photosynthesis in the deteriorated phytoplankton, although the distinction between the real shade form and the deteriorated one is rather difficult only through the characteristics of photosynthesis curve.

With the beginning of the circulation of water in autumn, the process of the differentiation of photosynthesis pattern will be repeated. Usually, the differentiation of photosynthesis pattern in the vertical direction did not developed definitely in shallow lakes as the result of the complete circulation of water.

Summary

Photosynthesis pattern relating to light intensity and the process of the differentiation in pattern have been pursued in natural phytoplankton community of lakes.

1. Phytoplankton growing in a lake can be classified into the sun and shade forms through the characteristics in photosynthesis-light curve. The typical sun form phytoplankton is usually obtained in the surface layer phytoplankton in early summer and autumn, and the phytoplankton taken from deeper layer acts as the shade form. During late summer and winter, all phytoplankton in a lake indicates the shade type photosynthesis.

2. The photosynthesis pattern differs with difference of species but the difference is rather slight under the optimal environmental condition for each species. Regional difference in photosynthesis pattern can be observed definitely. The phytoplankton acts as a sun form in enriched water of eutrophic lakes and a shade form in poor water of oligotrophic lakes.

3. The factors relating to the differentiation of photosynthesis pattern, i.e. light, temperature and nutrients, are ascertained experimentaly. However, the effect of past history of environmental factors on the photosynthesis pattern does not hold for a long period and the pattern rapidly transforms through the change of environmental factors.

4. The differentiation of photosynthesis pattern directly refers to the movement of water in lakes. During the circulation period, all phytoplankton in a photic layer show the sun-type photosynthesis pattern and with stagnating of water the photosynthesis pattern differentiates into the sun type in the surface phytoplankton and the shade one in the deep-layer phytoplankton.

The author wishes to express his cordial thanks to Prof. M. Monsi and Prof. K. Hogetsu, under whose guidance this research has been carried out.

References

1) Talling, J. F., The New Phytologist **56**: 29 (1957). 2) Rodhe, W., Vollenweider, R. A., and Nauweek, A., In Perspectives in Marine Biology, A. A. Buzzati-Traverso, Ed., U. of Calif. Press, Berkeley (1958). 3) Steemann Nielsen, E., Physiologia Plantarum **12**:353 (1959). 4) Ryther, J. H., and Menzel, D. W., Limnol. and Oceanography **4**: 492 (1959). 5) Ichimura, S., and Saijo, Y., Bot. Mag. Tokyo **71**: 174 (1958). 6) Gessner, F.. Schweizerische Zeitschrift für Hydrologie **11**: 378 (1949). 7 Ichimura, S., Bot. Mag. Tokyo **71**: 112 (1958). 8) Sano, K., and Matsue, Y., Suisan-Zoshoku **6**: 10 (1958). 9) Ryther, J. H., Limnol. and Oceanography **1**: 61 (1959). 10) Ichimura, S., and Aruga, Y., Bot. Mag. Tokyo **71**: 263 (1958). 11) Verduin, J., Ecology **37**: 40 (1956). 12) Edmondson, W. T., and Edmondson, Y. H., J. Mar. Res. **6**: 228 (1947).

摘　要

市 村 俊 英:　植物プランクトンの陽生型および陰生型光合成

植物プランクトンの光合成の特性を明らかにすることは，水界の基礎生産の解析のため，きわめて重要である．本研究では特に自然植物プランクトンに見られる陽生型および陰生型の光合成について論じた．

一般に夏季，秋季のプランクトンの光合成は陽生型であり，冬季のプランクトンでは陰生型である．

垂直分布による光合成型の分化は特に夏季停滞期に明瞭に見られ，表層プランクトンでは陽生型，深層プランクトンでは陰生型を示す．循環期にはいちじるしい分化は見られない．また富栄養湖では陽生型，貧栄養湖では陰生型の光合成が得られる．これら光合成型は種固有のものでなく環境条件の変化によって短時間で容易に他の型に変化する．分化を促進する条件とし，停滞による湖沼内プラントクンの垂直的な受光量の相違，季節的な水温の変化，栄養塩類傾度が実験的に確かめられた．これらの結果にもとづいて季節的な陽生，陰生両光合成型の分化過程を明らかにした．（東京教育大学理学部植物学教室）

Bot. Mag. Tokyo, **73**: 468—473 (December 25, 1960)

Studies on the Light Controlling Flower Initiation of *Pharbitis Nil.* VIII. Light-Sensitivity of the Inductive Dark Process

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

Received June 17, 1960

In previous papers[1-5]), it was reported that in *Pharbitis* seedlings the first phase of the inductive dark period was relatively light-stable and that the last phase of the 16-hour dark period (12th—16th hour) was also relatively stable to the light but not so much as the first one.

Light-sensitivity of the inductive dark process is considered to vary with time during the dark period. In the present investigation, the dark process was divided into several phases, and the light-sensitivity of each process was investigated.

Material and Methods

Seedlings of *Pharbitis Nil*, strain "Violet", were used as material. Procedures of experiments were similar to those described in a previous paper[6]). To secure monochromatic light, interference filters combined with coloured glass were used. Spectral transmittances of these filters were also described in the previous paper[6]).

A 16-hour dark period which induces a maximum flowering response in *Pharbitis* seedlings was divided into 4 phases, each consisting of 4 hours. These phases may be characterized as follows:

1st phase: relatively light-stable: a brief light interruption given during this phase does not inhibit the flowering response[1,2,7]).

2nd phase: a brief light interruption given during this phase inhibits flower initiation to some extent[7]). One 8-hour dark period consisting of the 1st and 2nd phases is subcritical for flower initiation and does not induce flowering even if it is given repeatedly[8]).

3rd phase: a brief light interruption given during this phase strongly inhibits the flowering response[7]). One 12-hour dark period consisting of the 1st, 2nd and 3rd phases can induce flowering when given repeatedly, but is critical for flower initiation when given once[2,8,9]).

4th pahse: a brief light interruption given during this phase inhibits flower initiation to some extent[7]). One 16-hour dark period consisting of the 1st, 2nd, 3rd and 4th phases induces a maximum flowering response and further lengthening of the dark period does not increase the flowering response[2,8]).

Plants were subjected to light of various intensities or qualities during each phase. They were kept in darkness during the remaining 3 phases.

Experiments and Results

Experiment 1. Plants were divided into 4 groups. The first group consisting of 6 lots of 40 plants each was subjected to daylight fluorescent lights of 500, 200, 100, 50, 10 and 1 lux during the first phase. The 2nd, 3rd and 4th groups each consisting

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

of 6 lots were also subjected to these lights during the 2nd, 3rd, and 4th phases, respectively. During the remaining 3 phases, all plants were kept in darkness. Control plants were subjected to dark periods of 12 or 16 hours.

Table 1. Light-sensitivity of the dark process in the 1st, 2nd, 3rd and 4th phases of a 16-hour dark period. Each phase consisted of 4 hours duration. Plants were subjected to daylight fluorescent light of 1–500 lux during the 1st, 2nd, 3rd or 4th phase and kept in darkness during the remaining 3 phases. (Treated on June 22 and dissected on July 6, 1959)

Phase subjected to the light	Intensity of the light (lux)	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
I	500	37	100	3.1	0
	200	36	100	4.3	63.9
	100	36	100	4.9	97.2
	50	37	100	4.5	100
	10	37	100	4.8	100
	1	38	100	4.6	97.4
II	500	38	0	0	0
	200	38	21.0	0.2	0
	100	37	89.2	1.3	0
	50	38	94.8	2.6	2.6
	10	38	100	3 7	18.4
	1	35	100	5.0	80.0
III	500	37	0	0	0
	200	38	0	0	0
	100	37	0	0	0
	50	38	0	0	0
	10	38	0	0	0
	1	36	55.6	1.2	0
IV	500	37	0	0	0
	200	38	0	0	0
	100	36	2.8	0.0	0
	50	38	50.0	0.7	0
	10	37	83.8	1.7	0
	1	36	100	3.7	25.0
Control 12^h d		36	0	0	0
Control 16^h d		37	100	4.8	97.3

12^h d: 12-hour dark period
16^h d: 16-hour dark period

Flowering responses are shown in Table 1. None of the plants subjected to uninterrupted darkness for 12 hours (Control) initiated flower primordia, but all the plants subjected to light of 500 lux during the first phase and to darkness during the remaining 12 hours initiated flower primordia. Thus, the process taking place during the first phase appears to be very stable to the light.

Intensities of the light under which about 50 per cent of the plants initiated flower primordia were 100—200 lux in the 2nd phase, 1 lux in the 3rd, and 50 lux in the 4th phase. The 3rd phase is most sensitive to the light, the light-sensitivity of the 4th, 2nd and 1st phases decreasing in the order named.

Experiment 2. The first phase of the inductive dark period appears to be very stable to light. To investigate whether or not the light-sensitivity of the 1st phase

is influenced by the light-intensity preceding it, the following experiments were undertaken.

Plants were divided into 3 groups, and each group was subjected to the following light conditions for a 12-hour period.

1. Bright sunlight (20,000—100,000 lux)
2. Diffused natural daylight (Plants were placed in the shade; 3,000—6,000 lux)
3. Daylight fluorescent light of 1000 lux.

At the end of these treatments, plants of each group were divided further into 7 lots. The first five lots of all groups were subjected to 4 hours of daylight fluorescent lights of 2000, 1000, 500, 200 and 100 lux respectively, and thereafter to 12-hour darkness. The 6th and 7th lots were subjected to 12 and 16 hours of darkness respectively. Results are shown in Table 2.

Table 2. Effect of light-intensity of the pre-illumination upon light-sensitivity of the dark process in the first 4 hours of a 16-hour dark period.
Plants were subjected to bright sunlight (20,000—100,000 lux), diffused daylight (3000—6000 lux) or daylight fluorescent light of 1000 lux for 12 hours, and thereafter to 4 hours of daylight fluorescent light of 2000—100 lux followed by a 12-hour dark period.
(Treated on July 9 and dissected on July 23, 1959)

Pre-illumination	Intensity of light given during the first 4 hrs. (lux)	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
Bright sunlight	2000	35	8.6	0.1	0
	1000	34	55.9	0.6	0
	500	37	86.5	1.3	0
	200	35	91.4	1.5	0
	100	37	97.3	1.8	0
	Control 12^h d	37	0	0	0
	Control 16^h d	36	97.2	1.5	0
Diffused daylight	2000	36	83.3	1.1	0
	1000	34	97.1	1.2	0
	500	38	100	2.9	15.8
	200	36	100	4.8	91.7
	100	37	100	4.8	97.3
	Control 12^h d	38	15.8	0.2	0
	Control 16^h d	38	100	3.3	26.3
Daylight fluorescent light of 1000 lux	2000	35	68.6	0.7	0
	1000	35	88.6	1.2	0
	500	37	100	3.2	24.3
	200	32	100	4.2	56.6
	100	37	97.3	3.1	21.6
	Control 12^h d	37	97.3	1.1	0
	Control 16^h d	38	94.7	3.7	28.9

Plants subjected to a 12-hour dark period preceded by bright sunlight, diffused daylight, or fluorescent light of 1000 lux initiated 0, 0.2 or 1.1 flower buds per plant, respectively. That the plants subjected to a 12-hour dark period preceded by fluorescent light of 1000 lux initiated more flower buds than those pre-illuminated with bright sunlight or diffused daylight, may be attributable to the fact that the first process of the inductive dark period proceeds to some extent during the pre-illumination, i.e. during the 12-hour illumination with daylight fluorescent light of 1000 lux.

As a whole, plants pre-illuminated by bright sunlight initiated fewer flower buds than the others. For instance, the plants subjected to a 16-hour dark period preceded by bright sunlight initiated only 1.5 flower buds per plant, whereas those pre-illuminated with diffused daylight or daylight fluorescent light of 1000 lux initiated 3.3 or 3.7 flower buds per plant. Light of exceedingly high-intensity seems to be unfavourable for photoperiodic induction of *Pharbitis* seedlings, but details are unknown.

Light-stability of the first phase does not appear to be influenced by the intensity of pre-illumination. In all three groups, the first process is assumed to proceed to some extent under light of 2000 lux and to the same extent as in the darkness under light of 200 lux or less.

Experiment 3. Spectral sensitivities of the 4 phases were investigated. Plants were subjected to coloured light during the 1st, 2nd, 3rd or 4th phase of a 16-hour dark period, and kept in darkness during the remaining 3 phases. The coloured lights used were violet (406 mμ), blue (489 mμ), red (649 mμ) and far-red (747 mμ); the spectral energy distribution of each is similar to that described in a previous paper[6]). Intensities of the coloured light at the leaf surface were adjusted to 1000, 100, 10 and 50 erg/cm.^{2}/sec. in the 1st, 2nd, 3rd and 4th phases, respectively. Control plants were subjected to a 12- or a 16-hour dark period preceded and followed by natural daylight.

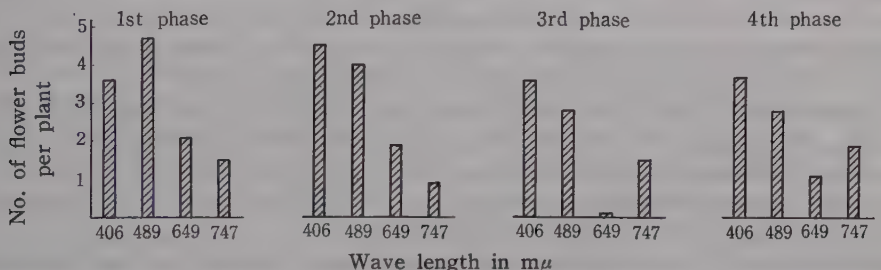


Fig. 1. Spectral sensitivity of the dark process in the 1st, 2nd, 3rd and 4th phases. Plants were subjected to coloured light during the 1st, 2nd, 3rd or 4th phase of a 16-hour dark period. Intensities of the coloured light were 1000, 100, 10 and 50 erg/cm.^{2}/sec. in the 1st, 2nd, 3rd and 4th phases, respectively.

One of the data is given in Fig. 1. Control plants subjected to a 12-hour dark period did not initiate flower buds, and those subjected to a 16-hour dark period initiated 4.8 flower buds per plant. Fig. 1 shows that the spectral-sensitivity of the dark process varies considerably with the phase of the dark period. During the 1st and 2nd phases far-red radiant energy is more effective than red. During the 3rd and 4th phases red radiant energy is more effective than far-red. Blue is less effective than violet in the first phase, but the reverse is the case for the other phases. In all phases of the dark period blue and violet are less effective than red or far-red.

Discussion

Light given during the inductive dark period inhibits the flower initiation of *Pharbitis* seedlings in various ways.

1) The light shortens the dark period, i.e. the light given during any phase of the dark period prevents the reaction which should proceed during that phase in darkness.

2) The light nullifies the dark process, i.e. it inhibits flower initiation even if a

dark period of sufficient duration follows it[7]).

Nullification of a dark process appears to take place in two ways:

i) One is based on a so-called "light-break effect", which is effective only during the first 6 to 14 hours of the dark period irrespective of the duration of the dark period[7]). The most effective portion of a spectrum for the "light-break effect" is red, and this flower inhibitory effect may be reversed by a following far-red irradiation[7,10,11]).

ii) The other is brought about by far-red irradiation, and we call this a "far-red effect" in the present paper. A brief far-red irradiation given during the first 12 hours of the inductive dark period inhibits flower initiation irrespective of the duration of the dark period[7]). Far-red given prior to the inductive dark period also inhibits flower initiation[2,3,6,7,9,12]). The mechanism of flower inhibition caused by far-red irradiation ("far-red effect") appears to differ from that of "light-break effect"[7]). The former is considered to bring about some changes or conditions which prevent the following dark process, and reversed by a subsequent irradiation with red. The latter is considered to destroy some substances (maybe precursors of flower-inducing substance) produced during the first hours of the dark period, and may be reversed by a subsequent far-red irradiation[7]).

These may be summarized as follows:

Light given during the inductive dark period has three effects, i.e. a) it shortens the dark period, b) gives a "light-break effect", and c) gives a "far-red effect".

Which effect is a predominant factor for suppressing flower initiation, depends upon the light quality and the phase during which light is given.

In Experiments 1 and 2, daylight fluorescent light which comprises little far-red was used, and it may not be necessary to consider the "far-red effect". "Light-break effect" appears to be a predominant factor for flower inhibition in Phase 3. In Phase 1, the effect of shortening a dark period may be predominant, but in Phases 2 and 4, it is uncertain which effect is predominant.

In Experiment 3, monochromatic light was used. In the first and the second phases, far-red was the most effective portion of a spectrum for flower inhibition. However, many experiments reported in previous papers[2,3,9]) suggested that the first phase and probably the second phase can proceed under far-red of relatively high intensity. It is assumed that far-red given during these phases has a "far-red effect" and prevents the following dark process, but scarcely inhibits the reaction which should proceed during these phases in darkness.

In the third phase, "light-break effect" may be a predominant factor for flower inhibition. Red light was the most effective. This agrees with the findings of Borthwick *et al.*[10,11]). However, blue light was less effective than violet, which does not agree with the results of Borthwick *et al.*

Except for far-red, red radiant energy is most effective throughout the inductive dark period for suppressing the dark process. An interesting fact is that the blue light is less effective than violet in the first phase, but more effective than violet in the other phases. The first phase is very light-stable and the spectral sensitivity also differs from the others. Light given during the first 4 hours of the inductive dark period has no "light-break effect". This may be the reason why the first process differs from the others.

Summary

An inductive dark period of 16 hours was divided into 4 phases consisting of 4 hours each.

1) Light-sensitivity of these 4 phases was investigated. The first phase was most stable to the light, the light-stability of the 2nd, 4th and 3rd phases decreasing in the order mentioned. The first phase proceeded to some extent under daylight fluorescent light of 2000 lux, but the 3rd phase did not proceed under light of 10 lux.

2) Light-stability of the 1st phase was not influenced by the light-intensity preceding it.

3) Spectral sensitivity of the inductive dark process varied with the phase.

During the 1st and 2nd phases, far-red radiant energy was more effective than red. During the 3rd and 4th phases, red was more effective than far-red. In the first phase, blue was less effective than violet, but the reverse was the case in the other three phases. In all phases, blue and violet were less effective than red or far-red.

Grateful acknowledgment is made to Professor S. Imamura for his suggestions and criticisms.

References

1) Takimoto, A., Ikeda, K., und Imamura, S., Bot. Mag. Tokyo **71**: 317 (1958). 2) Takimoto, A., and Ikeda, K., ibid. **72**: 388 (1959). 3) ——, and ——, ibid. **73**: 37 (1960), 4) ——, and ——, ibid. **73**: 91 (1960). 5) ——, and ——, ibid **73**: 175 (1960). 6) ——, and ——, ibid. **72**: 137 (1959). 7) ——, and ——, ibid. **73**: 341 (1960). 8) Kujirai, C., und Imamura, S., ibid. **71**: 408 (1958 . 9) Takimoto, A., and Ikeda, K., ibid. **72**: 181 (1959). 10) Parker, M. W., Hendricks, S. B., Borthwick, H. A., and Scully, N. J., Bot. Gaz. **108**: 1 (1946). 11) Borthwick, H. A., Hendricks, S. B., and Parker, M. W., ibid. **110**: 103 (1948). 12) Nakayama, S., Sci. Report of Tohoku Univ., S4, Biol. **24**: 137 (1958).

摘　　要

滝本　敦・池田勝彦:　アサガオの花芽形成を支配する光条件について
VIII.　暗期反応の光感受性

アサガオに 16 時間の暗期を一度与えると，充分な花芽形成が起こる．この 16 時間の暗期を 4 時間づつの 4 つの Phase にわかち，おのおのの光感受性を調べた．

1) 最初の Phase すなわち Phase I が最も光に安定であり，Phase II, IV, III, がこれに続く．Phase I は 2000 ルックスの昼光色螢光燈下でも多少進行しうるが，Phase III は 10 ルックスの昼光色螢光燈下で全く進行し得ない．

2) Phase I の光感受性はそれに先行する光の強さに左右されない．

3) 暗期反応の光感受性に対する波長特性は，その Phase によって異なる．Phase I および II においては近赤外光が最も有効に働き，赤色光がこれに次ぐ．Phase III および IV においては，赤色光が最も有効であり，近赤外光がこれに次ぐ．Phase I においては青色光が紫色光よりも有効であるが，他の Phase においてはこれが逆になる．すべての Phase を通じて青および紫色光は赤および近赤外光より効果が少ない．(京都大学農学部応用植物学研究室)

Bot. Mag. Tokyo, **73**: 474–478 (December 25, 1960)

The Spindle of the Yeast-Cell

by Akira YUASA*

Received June 22, 1960

The opinion that a yeast-cell contains a normal nucleus which is observed in the higher plant has been advocated by various authors [Guilliermond (1910)[5]), Kater (1927)[9]), Beams, Zell and Sulkin (1940)[1]), Sinotô and Yuasa (1941)[21]), Yuasa (1958)[27]), Swaminathan (1958)[22], Hilde and Douglas (1957)[8]) etc.]. On the other hand, the so-called vacuolar nucleus has also been advocated by some authors [Wager (1898)[25], Wager and Peniston (1910)[26]), Marpamn (1908)[15]) etc.]. Lindegren (1951, 1951)[10,11]), and Townsend and Lindegren (1953)[24]) has the opinion that the spindle (the nucleus of Guilliermond and others) contains the chromosome and the chromosomes appear in the vacuole during the course of nuclear division.

The mode of the nuclear division of the yeast-cell has also been discussed by many investigators. Amitosis was observed by Guilliermond (1910)[5]), Beams, Zell and Sulkin (1940)[1]) and others. Hashimoto, Conti and Naylor (1959)[6]) and Hashimoto and Naylor (1960)[7] also thought, using the electron-microscope, that the nucleus divided by amitotic process. Mitosis was advocated by Kater (1927)[9]), Sinotô and Yuasa (1941)[21]), DeLamater (1950)[2]), Lindegren, McClary and Williams (1955)[14]), McClary, Williams and Lindegren (1957)[16]), Yuasa (1958)[27]) and Swaminathan and Ganessan (1958)[23]).

During the course of nuclear division, the centrosome or centriole was observed by Ranganathan and Subramaniam (1947)[18]), Subramaniam (1951)[22]), DeLameter (1950)[2]), Swaminathan and Ganessan (1958)[23]) and Yuasa and Lindegren (1958)[28]).

One of the reasons that the situation of nucleus and nuclear division has not yet been clarified is owing to the fact that the membrane of a yeast-cell has the special composition and that the fixation and staining of the yeast-cell is difficult to accomplish. According to Northcote (1954)[19]) the cell-membrane of the yeast-cell is composed of galactan, mannan, lipid and proteid.

So the present writer tried, at first, to get the good fixation and staining of the yeast-cell by various methods in order to make the structure of the cell clear.

The present report is the results of the study on this problem.

Materials and Methods

The materials are the cells of *Saccharomyces cerevisiae* "Rasse" whose ploidies are uncertain. The cells were cultivated in the "Koji"-solution.

The cells were fixed and stained 6 hr after the cultivation. The methods of fixation and staining were shown, in detail, in the following chapter, respectively.

Results

1. Copper-ammonium solution

The cells were stained with Giemsa's solution after treated with copper-ammonium solution for 1 min.—3 hr. The cell-membrane is somewhat destroyed and deeply stained with Giemsa's solution, so the nucleus can not be observed clearly.

* Department of Biology, College of General Education, University of Tokyo, Tokyo, Japan.

2. Carnoy's fluid

Carnoy's fluid penetrates very rapidly into the cell through the membrane and fixes the nucleus. Therefore, the staining with acetocarmine-solution, Giemsa's solution or nucleus-staining dyes is very much effective when cells are fixed with Carnoy's solution.

Dilute solution of HCl or perchloric acid makes remove RNA, so the staining with nucleus-staining dyes is very good to stain the nucleus, when cells are fixed with Carnoy's solution and then treated with dilute solution of HCl or perchloric acid.

The contents of the vacuole of yeast-cell are different from those of the higher plants. Carnoy's fluid fixes the contents of the vacuole of yeast-cell rapidly and the form of the vacuole is reserved adequately, though the fixation of cytoplasm is not good.

3. Cell-wall lytic enzyme

The extract solution of cell-wall lytic enzyme was prepared according to Furuyama and Ikeda's method (1960)[4]. The yeast-cells were treated with this extract solution for 5—60 min., hydrolyzed with 1 N-HCl for 5—15 min. at 60° and stained with Giemsa's solution. The nucleus was stained clearly, located near the vacuole. The nucleus is positive to Feulgen nucleus staining reaction.

The various nucleus-staining dyes (carmine, gentian violet, methyl-green, safranine and thionin) can stain the nucleus clearly after the above-mentioned treatment.

4. Benda's solution

Benda's solution is a good fixative to fix cytoplasm. To get the good figure of vacuole, Benda's solution was used and then Carnoy's fluid is used to make the staining dye penetrate rapidly. Perchloric acid-Giemsa's staining was adopted.

The nucleus was not observed clearly. The cytoplasm stains deeply, so the vacuole can not be seen obviously.

Sometimes the cells were fixed with Benda's solution after the pretreatment with Carnoy's fluid and then stained according to the above-mentioned technique. But, the result was not good.

5. Schaudinn's solution

Schaudinn's solution is a fixative which fixes the cytoplasm considerably well. In this case, the staining is very faint.

6. Champy's solution and Flemming's solution

When yeast-cells were treated with the cell-wall lytic enzyme, hydrolyzed with 1 N-HCl and stained with Heidenhain's iron-alum haematoxylin the nucleus and vacuole were reserved adequately, but many granules in the cell stain deeply and hinder the observation of the nucleus.

7. 45% acetic acid

45% acetic acid is a good fixative which fixes the nucleus and vacuole. The nucleus can be stained clearly with the solution of toluidine blue or Giemsa's staining dye after the fixation with Carnoy's fluid.

45% acetic acid can be recommended as one of the good fixatives of nucleus.

8. Cadmium chloride

1% $CdCl_2$ is a good fixative of the spindle. In this study, cells were suspended in 1% aqueous solution of $CdCl_2$ and observed by phase-contrast microscope.

Spindle can be observed somewhat clearly and stained with the solution of toluidine blue.

The nucleus changes to spindle and the chromosomes appear in the spindle when the nucleus divides [cf. McClary, Williams, Lindegren and Ogur (1957)[17], Lindegren and Lindegren (1951)[13]]. This process of mitosis was traced in this study by electron-microscopy.

The cells were treated with cell-membrane lytic enzyme, hydrolyzed with 1 N–HCl for 7 min. at 60° and stained with various spindle-staining dyes (aniline blue, erythrosine, fast green FCF, methylgreen, light green, malachite green, basic fuchsin, toluidine blue or eosin). In the case of toluidine blue, spindle can be stained, but in the cases of other dyes spindle can not be stained.

9. Vital staining

The cells were vitally stained with various solutions of staining dyes (trypan blue, trypan red, Isamine blue, neutral red, Janus green, brilliant cresyl blue, methylene blue, Bismarck brown etc.). The cells were cultivated in the "Koji" solution which contained the vital staining dyes in concentration of 0.01, 0.001, 0.0001%.

The number of the cells after the cultivation of 24 hours is as follows:

Vital staining dyes	Concentration Initials	0	0.0001%	0.001%	0.01%
Control	7.2×10^8	9.0×10^9	—	—	—
Neutral red	7.2×10^8	—	8.5×10^9	7.1×10^9	6.9×10^9
Janus green	7.1×10^8	—	8.1×10^9	8.2×10^9	7.9×10^9
Methylene blue	7.2×10^8	—	8.5×10^9	8.3×10^9	9.2×10^9
Bismarck brown	7.2×10^8	—	8.7×10^9	7.8×10^9	8.3×10^9

In this case, only several~16% of the cells are stained and the remaining cells are not stained. The cause of unstainability is not certain and remained to the future study.

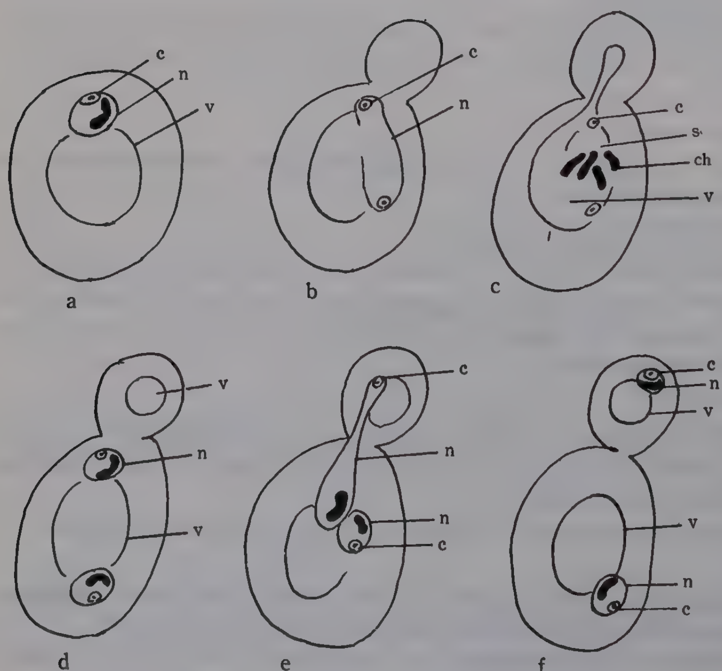


Fig. 1, a–f. Schematic representation of mitosis of yeast-cell.
c, centrosome;
ch, chromosome;
n, nucleus,
s, spindle;
v, vacuole
a, resting nucleus is seen. b, nucleus changes its form. c, nucleus changes to spindle and chromosomes appear. d, two daughter nuclei are seen. e, f, one of the daughter nuclei enters into the bud.

Discussion

One of the reasons why there are many opinions about the structure of nucleus, chromosomes and mitotic apparatus in yeast-cells is that the cell-membrane has specific composition and there are some difficulties to fix and stain the chromatic substance and achromatic materials.

Northcote (1954)[19]) confirmed that the cell-membrane of the yeast-cell is composed of glucan, mannan, lipid and protein. Eddy and Rudin (1927)[3]) also stated that the cell-membrane of yeast-cell contains galactan, mannan, lipid and proteid.

Therefore it is thought that adequate methods should be considered to get good fixation and staining of the yeast-cells. Carnoy's fluid and 45% acetic acid can penetrate quickly into the cell and fix the chromatinic substance adequately. In this case the cell-membrane shows no obstruction to the fixation.

Carnoy's fluid can also fix the contents of the vacuole which seems to be the specific substances. This fixative can not fix the cytoplasm adequately, but preserve the vacuole well.

The cell-wall lytic enzyme which has been discovered by Furuya and Ikeda (1960)[4]) is effective. The cells which have been treated with this enzyme can be fixed and stained considerably well.

The hydrolysis with HCl or perchloric acid is also effective in taking off RNA of the cell and bringing good results to the staining.

Therefore, the treatment with cell-wall lytic enzyme, fixation with 1 N–HCl for several minutes and then staining with aceto-carmine solution, Giemsa's solution or the solution of nuclear staining dyes is the most effective method to observe the nuclear materials.

The nuclear material which was shown by this method is also positive to Feulgen's nuclear staining reaction.

The fixation of vacuole of the yeast-cell is difficult, but Carnoy's fluid, 45% acetic acid or Flemming's solution can fix the contents of the vacuole considerably well, though the fixation of cytoplasm is not effective by these fixatives [cf. Lindegren and Rafalko (1950)[12])].

To get the good fixation of the spindle, Satô (1959)[20]) used the solution of $CdCl_2$ in his electron-microscopical study of the cell of higher plants. This fixative is also effective in yeast-cells. The present writer wants to recommend the fixation with solution of $CdCl_2$ and then the observation with phase contrast microscope to observe the spindle of the yeast-cell.

Hashimoto, Conti and Naylar (1959)[6]) studied the process of mitosis of yeast-cell by electron-microscopy. They showed a good figure of nuclear division and seemed to have the opinion that the nucleus divides amitotically.

The present writer also studied the process of mitosis in yeast-cell by electron-microscopy and compared with that which was fixed and observed by phase-contrast microscope.

The detail will be published in the next paper, but it is thought that the nucleus changes to spindle of the mitosis in which chromosomes appear, overlapping the vacuole.

The figure which was thought to be amitosis is the stage in which one of the daughter nuclei after mitosis is about to enter the bud.

The vital staining of the yeast-cell shows an interesting fact that only several～16% of the cells stain, and the remaining ones do not stain. The reason is not clear

now and it must be remained to the future study

Summary

1. The Carnoy's fluid and 45% acetic acid are good fixatives to fix the nuclear material of yeast cells.

2. The method in which the treatment with cell-wall lytic enzyme, the fixation with Carnoy's fluid, hydrolysis with 1 N–HCl at 60° and staining with aceto-carmine or Giemsa's staining are used subsequently is very effective to observe the nuclear material.

3. The solution of $CdCl_2$ is effective to fix the spindle of the yeast-cell.

4. Carnoy's fluid, 45% acetic acid and Flemming's solution can fix the contents of the vacuole fairly well.

5. The nucleus changes to the spindle when the mitosis begins and the chromosomes appear in the spindle, overlapping the vacuole.

The present writer wishes to express his cordial appreciation to Dr. S. Itagaki and Mrs. M. Osumi for their assistance in this study.

References

1) Beams, H. W., Zell, L. W., and Sulkin, N. M., Cytologia **11**: 30 (1940). 2) DeLamater, E. D., Jour. Bact. **60**: 321 (1950). 3) Eddy, A. A., and Rudin, A. D., Jour. Gen. Microbiol. **17**: V (1957). 4) Furuya, A., and Ikeda, Y., Jour. Agr. Chem. Soc. Japan **34**: 33 (1960). 5) Guilliermond, A., Centralbl. f. Bakt. II, **26**: 577 (1910). 6) Hashimoto, T., Conti, S. F., and Naylor, H.B., Jour. Bact. **76**: 416 (1959). 7) ——, ——, and ——, ib. **77**: 343 (1959). 8) Hilda, D. A., and Douglas, H. C., Jour. Bact. **73**: 365 (1959). 9) Kater, J. McA., Biol. Bull. **52**: 436 (1927). 10) Lindegren, C. C., Exp. Cell Res. **2**: 305 (1951). 11) ——, ib. **2**: 275 (1951). 12) ——, and Rafalko, M. M., ib. **1**: (1950). 13) —— , and Lindegren, G., Jour. Gen. Microbiol. **5**: 885 (1951). 14) ——, McClary, D. O., and Williums, M. A., Cytologia **20**: 185 (1955). 15) Marpmann, G., Centralbl. f. Bakt. II, **9**: 357 (1902). 16) McClary, D. O., Williams, M. A., and Lindegren, C. C., Jour. Bact. **73**: 754 (1957.) 17) ——, ——, ——, and Ogur, M., ib. **73**: 360 (1957). 18) Ranganathan, B., and Subramaniam, M. K., Sci. and Cult. **12**: 478 (1947). 19) Northcote, D. H., Jour. Gen. Microbiol. **11**; VIII (1954). 20) Sato, Sy., Cytologia, **24**: 98 (1959). 21) Sinotô, Y., and Yuasa, A., ib. **11**: 464 (1941). 22) Subramaniam, M. K,, Proc. Nat. Inst. Sci. India **14**: 315 (1951). 23) Swaminathan, M. S., and Ganessan, A. T., Nature **182**: 610 (1958). 24) Townsend, G. F., and Lindegren, C. C., Cytologia **18**: 183 (1953). 25) Wager, H., Ann. Bot. **12**: 499 (1898). 26) ——, and Peninston, A., ib. **24**: 45 (1910). 27) Yuasa, A., Bot. Mag. Tokyo **71**: 275 (1958). 28) ——, and Lindegren, C. C., Antonie van Leeuwenhoek **25**: 73 (1958).

摘　　要

湯 浅　明: コウボキンの紡錘体

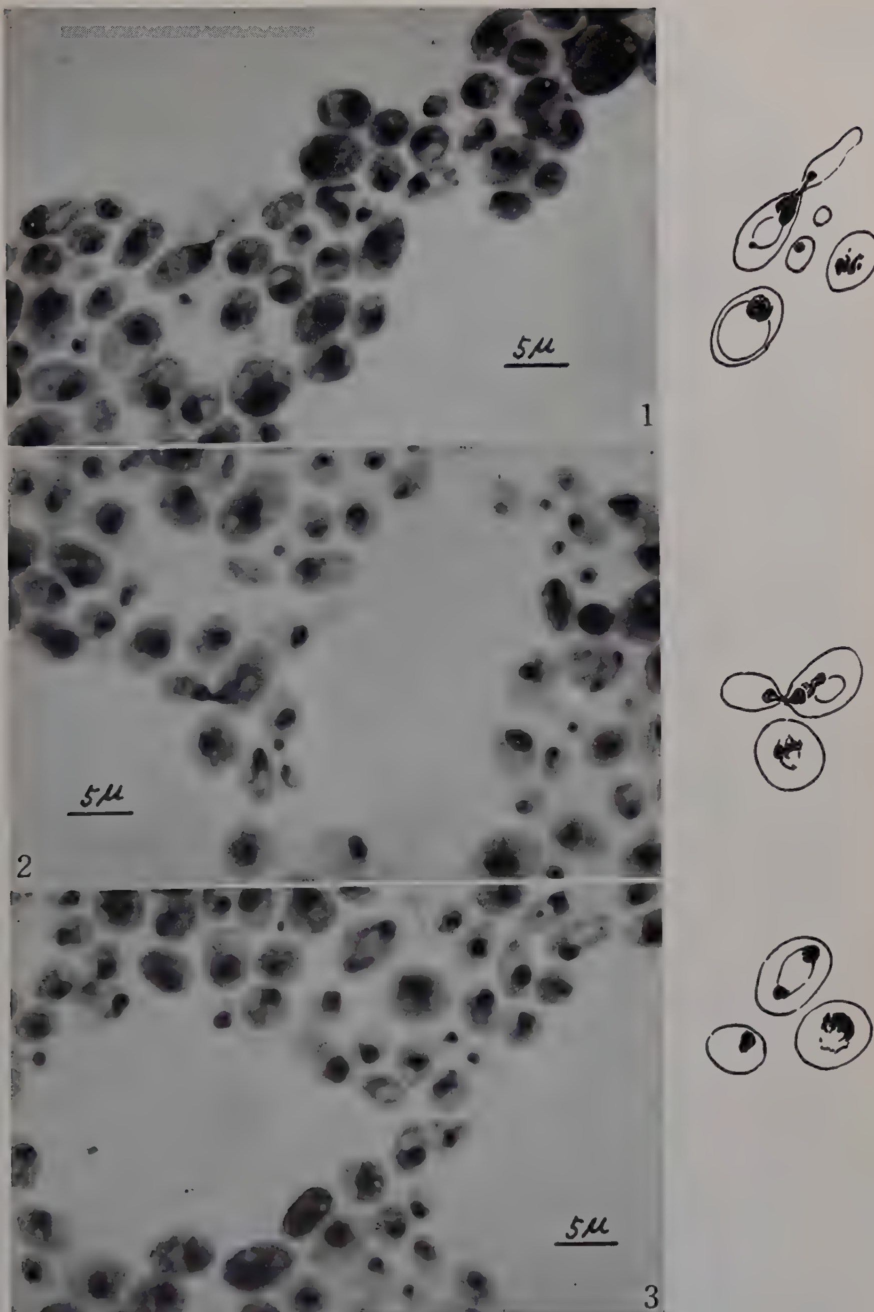
1. カルノア液，45%酢酸は，コウボキンの核質を固定するのに適している．

2. 細胞膜をとかす酵素でコウボキンを処理し，カルノア液で固定，60°で 1 N 塩酸で加水分解してから，酢酸カーミン液あるいはギームザ液で染色すると，核質を観察するのによい．

3. 塩化カドミゥムの 1% 水溶液は，コウボキンの紡錘体固定に有効である．

4. カルノア液，45%酢酸，フレミング液は，液胞の内容をよく固定する．

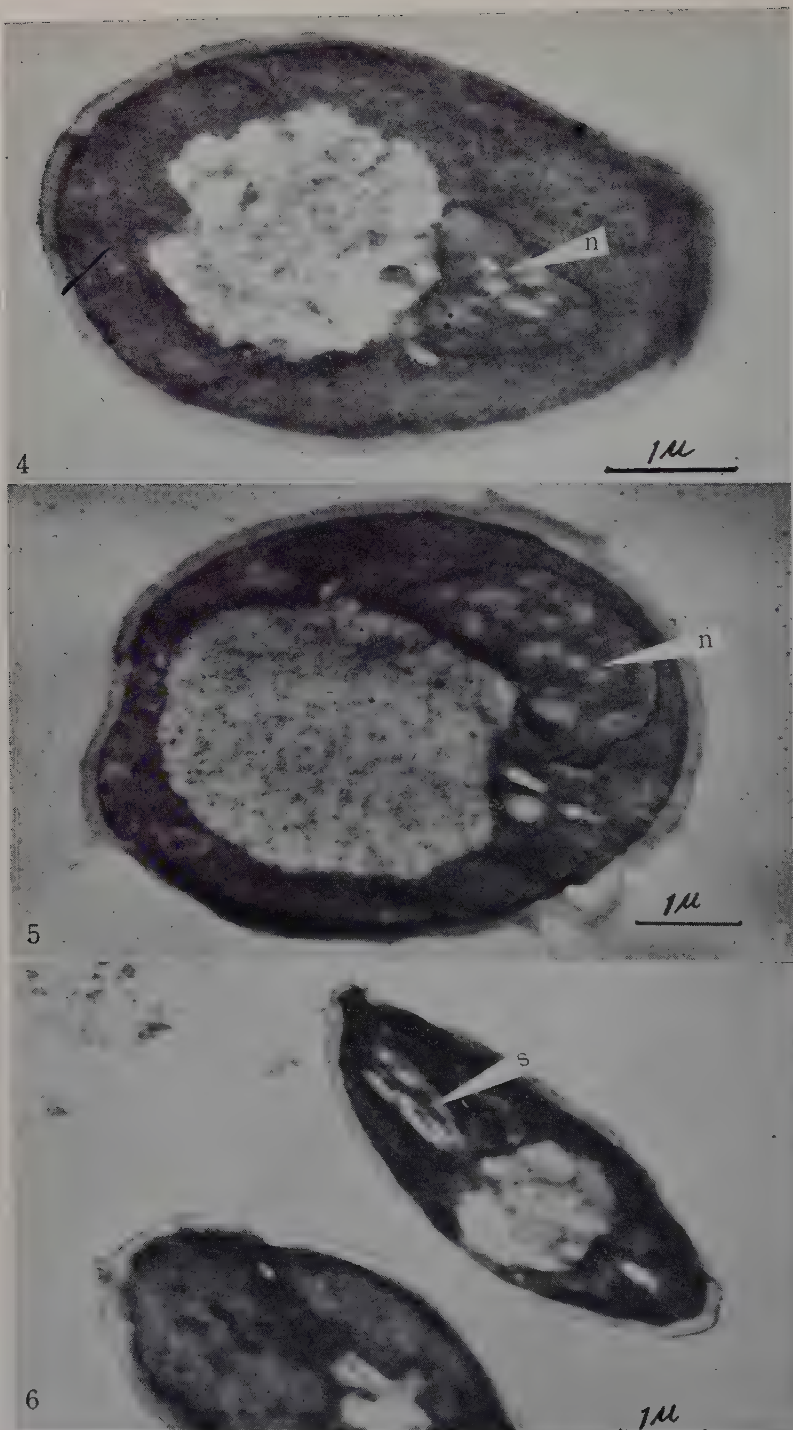
5. 核分裂がはじまると，核は紡錘体にかわり，染色体が現われるが，液胞と重なる位置である． (東京大学教養学部生物学教室)



Figs. 1–3. Carnoy's fluid–1 N HCl-Giemsa staining, after the treatment with cell-wall lytic enzyme. ×ca. 2800. 1, metaphase and the stage in which one of the daughter nuclei enters into the bud. 2, the same. 3, telophase and two daughter nuclei.

A. YUASA: The Spindle of the Yeast-Cell.

Plate 2.



Figs. 4-6. Electron-microscopical photographs. n, nucleus; s, spindle; 4, nucleus is clearly shown. 5, 6, nucleus changes to spindle.

A. YUASA: The Spindle of the Yeast-Cell.

Bot. Mag. Tokyo 73 : 479-482 (December 25, 1960)

マルバアサガオの日長反応におよぼす鉄不足の影響

木 下 哲 雄*・柴 田 治*

Tetsuo KINOSHITA* and Osamu SHIBATA*: On Photoperiodic Responses of *Pharbitis purpurea* Voigt as Influenced by Various Levels of Iron.

1960 年 1 月 11 日受付

植物栄養元素の過不足が花芽形成におよぼす影響についてはすでに数多くの研究があり，個々の元素またはそれらの相互の関係についてもかなり詳細に報告されている．しかし，鉄も含めて微量栄養元素に関するこの種の研究[1,2]はごくわずか報告されているにすぎない．組織学的な変化のうえから花芽形成期における硼素の働きについて，Struckmeyer[3]が多くの実験結果を報告しているが，これらの間に直接の関係を見いだすことはできなかった．

ここで研究の対象とした鉄は葉緑素の生成に欠くことのできないものであり同時に多くの酵素成分として有機物の代謝に重要な働きが認められている．それゆえ，鉄不足といってもその不足の程度によって生理的に種々の異なった状態が生じてくるはずである．Smith ら[2]は鉄不足で黄化したオナモミを用いて，これが日長反応におよぼす影響について報告しているが，筆者らがここでえた結果はたとえ鉄不足でも黄化していない植物についてのものである．この点 Smith らの場合と明らかに異なっている．

材料と方法

種皮の一部に傷をつけたマルバアサガオの種子をペトリー皿中で純水を与えて発芽させた．30° 前後では種子は 2 日間ぐらいで一斉に発根し，さらにその後 2 日間ほどで子葉が完全に展開する．子葉の展開した植物はその後クノープ液で培養したが，クノープ液中の $FeCl_3 \cdot 6H_2O$ のみは必要に応じて 9, 5, 1, 0.1 および 0.01 mg/l とした．ここに記した鉄量は結晶水を含んだ塩化物のままの重量ゆえ，実際の量としてはこれよりかなり少ないはずであるが，以下便宜上これを鉄量として記してゆくことにする．また 9 mg/l の鉄を与えた群はすべての場合に対照区として扱ったが，これは通常クノープ液成分としては十分な鉄量を有する水耕液である．それゆえ，鉄量がこれより少ない場合には一般にこれらを鉄不足と称した．

これらの植物は播種以後はすべて昼光色螢光燈の補光によって 24 時間照明下で培養し，短日処理は 16 時間暗期と 8 時間明期の組合わせによって 3 回行なった．短日処理を行なった時期は第 1 葉がひらき，さらに 2 葉目がひらき始めたときで，子葉はすべて切除してから行なった．気温の変化のために多少の変動はあったが，発芽から短日処理開始まではほぼ 15 日間であった．

培養液のおのおのについて 1 回の実験に用いた個体数は 12〜20 である．

結　　果

はじめに，葉緑素量にはほとんど影響しない鉄量の範囲を検討した．

植物を 9, 5, 1, 0.1, 0.01 mg/l の鉄を含む培液で育て，水耕開始後10日間培養液をかえずにおくと 5 種の培液群のうち鉄量 0.1 mg/l のものは葉がやや黄化，0.01 mg/l 区のものは明らかに黄化したが他は完全に緑葉であった．7 日間ごとに培養液をかえた場合には鉄量 0.01 mg/l 区の植物のみがやや黄化したが，0.1 mg/l 区の植物はそれ以上の鉄を与えた区の植物と同程度の葉色を示した．植物体が黄化しないかぎりは各鉄量区の植物の生長量にはほとんど差はなかったが，やや軟弱になる感があった．これらの結果から，以下の実験では日長効果の発現のうえに葉緑素量の多少が影響しないように，水耕液は 7 日間ごとにかえ，鉄量 0.01 mg/l の区は実験からはぶくことにした．したがって，ここで

* 信州大学文理学部生物学教室

* Department of Biology, Faculty of Liberal Arts and Science, Shinshu University, Matsumoto, Japan.

行なったすべての実験では水耕液中の鉄量が異なっても植物は黄化せず，葉色も外観上すべて等しかった．

実　験　1.

種子の発芽から花芽数の測定まで同一鉄量で培養した場合の開花反応を第1表にまとめた．

花芽数は鉄量の減少に伴なってかなり減少し，鉄量 0.1 mg/*l* 区と 1 mg/*l* 区の間で1％，5 mg/*l* と 9 mg/*l* 区の間では 5％ の危険率で有意差が認められた．しかし，1 mg/*l* と 5 mg/*l* 区の間には有意差はなかった．

Table 1. Photoperiodic responses of *Pharbitis purpurea* cultured with equal iron-level during all the growth period.

Iron-level*	Number of plants observed	Induced plants	Average number of flower primordia
mg./*l*		%	
0.5	13	92.3	1.15
1	13	100	2.09
5	13	100	2.33
9 (control)	13	100	2.92

* The values show the weight of $FeCl_3 \cdot 6H_2O$.

実　験　2.

実験1の結果は培養期間のすべてを通じて同一鉄量とした場合であるが，この実験では短日処理前，短日処理中，あるいは短日処理後に与えた鉄量の多寡がおのおの日長反応にどのような影響を与えるかを調べるため，次のごとく処理を行なつた．

（A）　短日処理終了までは実験1と同様に種々の鉄量，それ以後はすべて 9 mg/*l* の鉄を与える。

（B）　短日処理前は種々の鉄量，処理期をふくめてそれ以後はすべて 9 mg/*l* の鉄を与える．

（C）　全生長期を通じて 9 mg/*l* の鉄を与える．(対照区)

これによってえられた結果は第2表にまとめた．

実験のA群では鉄量の減少につれて花芽数もかなり減少したが，B群では花芽数にほとんど影響を与えなかった．これらを相互に比較した統計的な結果は次のごとくであった．ただし，ここには短日処理終了後に鉄量 9 mg/*l* とした実験区をA，短日処理期をふくめてそれ以後を鉄量 9 mg/*l* とした区をB，対照区をCであらわし，それに付加した数字は鉄量 (mg/*l* 数) をあらわしてある．

A群内のおのおのの間には危険率1％で有意差があった．しかし，B群内で，さらにB群内のおのおのとCの間にも危険率5％で有意差は認められなかった．A—0.1 と B—0.1, A—5 と B—5, A—0.1 とCのおのおのではいずれも危険率1％で有意差あり，A—1 とCには5％でも有意差なく，A—5 とCでは A—5 の方が5％の危険率で有意に増加していた．

実　験　3.

実験2でえられた結果をもとにして鉄不足と光合成生産物との関係をしらべるため，実験1と同様の水耕をしつつ，次の各期に 0.5％ glucose を根から吸収させた．（A）短日処理前 73 時間，（B）短日処理期間中 72 時間，（C）短日処理後 73 時間．ここで短日処理前と処理後に glucose を与える期間は短日処理期間の時間数になるべくちかく，また他の実験操作上不都合でない時間としてえらんだものである．

Table 2. Photoperiodic responses of *Pharbitis purpurea* cultured with different iron-levels during the different period of photoperiodic induction.

Iron-level*: Before photoperiodic induction	Iron-level*: During photoperiodic induction	Iron-level*: After photoperiodic induction	Number of plants observed	Induced plants	Average number of flower primordia
mg./*l*	mg./*l*	mg./*l*		%	
0.1	0.1	9	16	94	1.63
1	1	9	16	100	2.80
5	5	9	16	100	4.00
0.1	9	9	16	100	2.69
1	9	9	16	100	2.69
5	9	9	16	100	2.86
9	9	9 (control)	16	100	3.25

* The values show the weight of $FeCl_3 \cdot 6H_2O$.

Table 3. Photoperiodic responses of iron-deficient plants supplied with 0.5% glucose during the different period of photoperiodic treatment.

Period of supplying glucose	Iron-level*	Number of plants observed	Induced plants	Average number of flower primordia
	mg./l		%	
Before photo-induction (for 73 hrs.)	0.1	18	100	3.71
	1	18	100	4.72
	5	18	100	7.16
	9	18	100	7.50
During photo-induction (for 72 hrs.)	0.1	18	55.6	2.11
	1	18	94.4	3.78
	5	18	100	7.29
	9	18	100	7.33
After photo-induction (for 73 hrs.)	0.1	18	100	2.78
	1	18	100	2.89
	5	18	100	3.67
	9	18	100	3.72
No glucose	0.1	15	75	2.20
	1	15	77.8	2.73
	5	15	100	3.93
	9	16	88.9	4.63

* The values show the weight of $FeCl_3 \cdot 6H_2O$.

えられた結果は第3表にまとめた．短日処理後にglucose を与えた時の花芽数は鉄量 9 mg/l の場合，対照区のものより危険率1％で有意に少なかった．しかし，鉄量 0.1 mg/l の場合にはあまり差が認められず，むしろ逆に対照区の方が glucose を与えた区よりも花芽数は少なかった．

短日処理前あるいは処理期間中に glucose を与えた時には鉄不足でも花芽数はいちじるしく増加したが，この場合にも鉄量の差による違いがみられた．これら両期とも鉄 5 mg/l 区における花芽数の増加はことにいちじるしく，9 mg/l 区との間の有意差はなくなり，1 mg/l 区との間にはかなり顕著な差が生じた．

一般に鉄が低濃度の場合，glucose が短日処理前に与えられるといちじるしく花芽数を増加するが，短日処理中に与えた場合にはあまり顕著な影響を示さなかった．短日処理後に glucose を与えると，高濃度の鉄を与えた区では花芽数は減少するにもかかわらず，低濃度の鉄を与えた区では逆に花芽数は増加する傾向にあった．

考　　察

培養条件がかなり異なっているが，ここに記したと同様の実験は Smith ら[2]) がすでにオナモミで報告している．それによると，短日処理は植物体に鉄不足のきざしがあらわれてから緑葉をすべて切除して後に行なっており，その結果花芽形成は顕著に阻害されたという．筆者らがここに報告した場合は鉄不足といえどすべて正常な緑葉である．したがって，ここでえられた結果で Smith らのえた結果とは一致し難い場合もあったが，彼らの場合と同様に鉄不足が実験の全期にわたった時には花芽数が減少する，という結果においては一致していた．この花芽数は水耕液中の鉄量によっていちじるしく異なったが，このような花芽数の減少が日長反応の阻害によるものか，あるいは単なる発育阻害に基づくものであるかを断定することは困難である．

実験2の結果において，短日処理前の鉄不足は花芽数にはほとんど影響を与えず対照区との間にも差はなかったが，処理期間およびそれ以前がともに鉄不足であった時には花芽数はかなり減少した．実験の全期にわたって鉄不足であった時の結果との比較から，鉄不足の影響は短日処理期間中または処理後においてのみいちじるしく，短日処理前の鉄不足は花芽形成に影響していなかったといいうる．短日処理終了までの鉄量が 5 mg/l でそれ以後の鉄量が 9 mg/l であったものでは花芽数は対照区（全期間を通じて 9 mg/l の鉄を与えたもの）よりわずかに増加したが，処理後も 5 mg/l しか鉄を与えなかったものは対照区より花芽数が減少し，その効果が全く逆であった．鉄量がこれより少ない場合には短日処理終了後の鉄量のいかんをとわず，花芽数は対照区のものより減少した．これらの結果から，日長の変化の感受または花成刺戟の形成の過程が鉄の働らきに依存する度合いは，花芽形成の場合よりはずっと少ないように思われる．筆者の1人は葉緑素量にほとんど差の生じない程度の鉄不足で生長した水稲が，鉄を十分に与えられたものと幼穂分化の時期に全く差はないが，その後の生長はおくれたと報告した[1])．またサンショウモで短日処理期間中の重金属酵素阻害が日長反応を強めると報じたが[4])，これらとの間

の関連の有無は明らかではない.

糖の吸収が日長効果を強めることはすでに報告されている[5]が，短日処理前と処理期間中の吸収では全く同一の傾向で花芽形成はいちじるしく促進された．しかし，処理後の場合には鉄の高濃度において花芽形成はかなり阻害された．この場合は短日処理後ゆえに，花芽形成物質の生成以後の過程で阻害されたものであろう．植物体の一部を短日処理するとき他部に非処理葉があれば，日長効果は弱められるという報告[6,7]があるが，それらとの関係は明らかではない.

鉄の低濃度の場合と高濃度の場合で与えた glucose の効果がやや異なったが，これはおのおのの鉄量によって糖が日長反応のみに影響を与えたのか，または基礎的な栄養代謝にも影響をおよぼしたのかという違いから生じたものであろう．しかし，その詳細な点についてはここではふれえない.

終りに，終始多大の御援助と御鞭達をいただいた本学の中山包教授に謝意を表する.

文　　献

1) Shibata, O., Bot. Mag. Tokyo **72**: 477 (1959). 2) Smith, H. J., McIlrath, W. J., and Bogorad, L., Bot. Gaz. **18**: 174 (1957). 3) Struckmeyer, B. E., Amer. Nat. **134**: 135 (1950). 4) 柴田 治，植雑，**73**: 120 (1960). 5) Lang, A., and Melchers, G., Planta **33**: 653 (1943). 6) Borthwick, H. A., and Parker, M. W., Bot. Gaz. **101**: 860 (1940). 7) 中山至大，生態研，**12**: 35 (1949).

Summary

Pharbitis purpurea Voigt was cultured on Knop's solution whose iron was deficient in various degrees without inducing chlorosis in leaves. Three short-day treatments consisting of 8-hour light and 16-hour dark periods were given about 15 days after the germination, and flowering responses were observed.

1) When iron was deficient throughout the period of plant growth, the effect of short day treatment was decreased with decreasing iron concentration.

2) Iron deficiency preceding the short day treatment gave no influence on the flowering responses, but that during and/or following the short day treatment gave significant influences. The moderate deficiency of iron (5 mg./l of $FeCl_3 \cdot 6H_2O$) given during the short day treatment increased flowering response to some extent, but reduced when the same iron-level was continued after the short day treatment.

3) It was considered that the flower initiation was inhibited not by iron deficiency during the period before the short day treatments, but by the iron deficiency after the short day treatment. The moderate iron-deficiency during the short day treatment may accelerate the flowering response.

4) Plants were cultured under iron-deficient condition throughout the total period, and glucose was supplied before, during or after the short day treatment. The glucose promoted flowering response when supplied before or during the short day treatment, but inhibited when supplied after the short day treatment. A remarkable flower-promoting effect of the glucose was obtained under the moderate iron-deficient condition.

Bot. Mag. Tokyo **73** : 483-486 (December 25, 1960)

震生湖における水生菌類の遊走子の季節的変化*

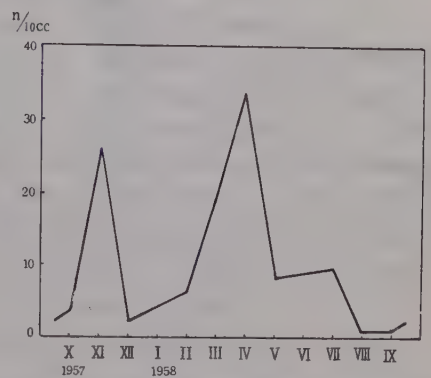
鈴　木　静　夫**

Shizuo SUZUKI**: Seasonal Variation in the Amount of Zoospore of Aquatic Phycomycetes in Lake Shinseiko

1960 年 4 月 8 日受付

湖沼植物プランクトンの季節的消長については，すでに多くの研究がなされているが，分解者として重要な働きをしている微生物群に関しては，細菌類を除いてほとんど知られていない．

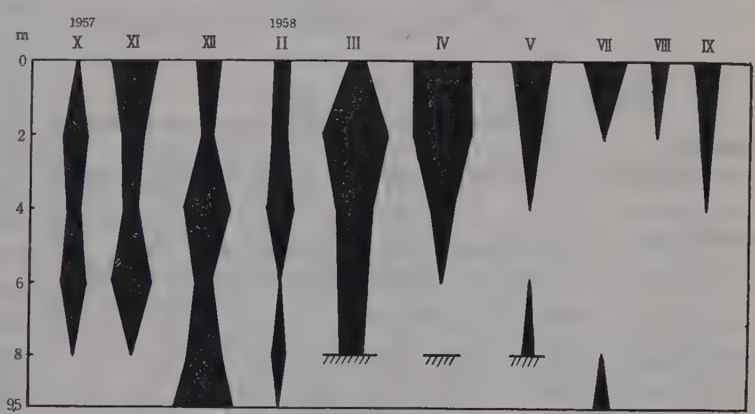
従来，湖沼の水生菌類の研究は，分類学的ならびにフロラ的研究にとどまり，生態学的な研究はわずかに Lund[1]), Forbes[2]), Weston[3]), Waterhouse[4]) の湖沼や河川の水生菌類を生態学的な見地より研究したのを見るにすぎない．著者は湖水中に存在する水生菌類の遊走子数を計測する方法を考案し，この方法を用いて震生湖において 1957 年 10 月より翌年 9 月にわたり遊走子の季節的変化を観察した．



第 1 図　水生菌類の遊走子数の季節的変化

研究方法

湖の湖心部において，各層の水を 2 m 間隔をもってエクマン式採水器を用いて採水し， 前報[5]) と同じ方法で試水中の水生菌類の遊走子数の計測ならびに種の同定を行ない，同時に水温，溶存酸素量を測定した．水温は転倒寒暖計，溶存酸素量は Winkler 法によって測定した．



第 2 図　水生菌類の遊走子の垂直分布の年変化

水生菌類の季節的変化

A．遊走子数の変化

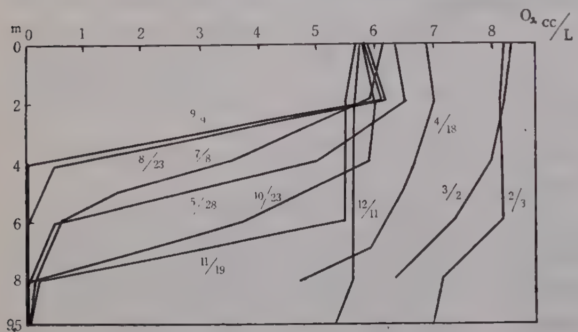
各層の遊走子数の平均値は第 1 図に示すような変化を示す．春の水温上昇に伴なって遊走子数は増加し，4 月に最大期に達し，その後の停滞期とともに減少し，8～9 月の水温の高い時期に最小値を示す．その後，水温の低下につれて増加し，11 月に 25/10 cc の最大を数えた．さらに水温低下に伴い急速に減少し，12～1 月に 2～3/10 cc の最小を示す．結局，2 回の最大期と，2 回の最小期が見られる．遊走子の垂直分布は第 2 図に示すように季節による二

* 本報の一部は日本植物学会第 24 回大会（仙台・1959）において報告した．

** Department of Microbial Chemistry, Faculty of Pharmacy, Tokyo College of Science. 東京理科大学薬学部微生物化学教室．

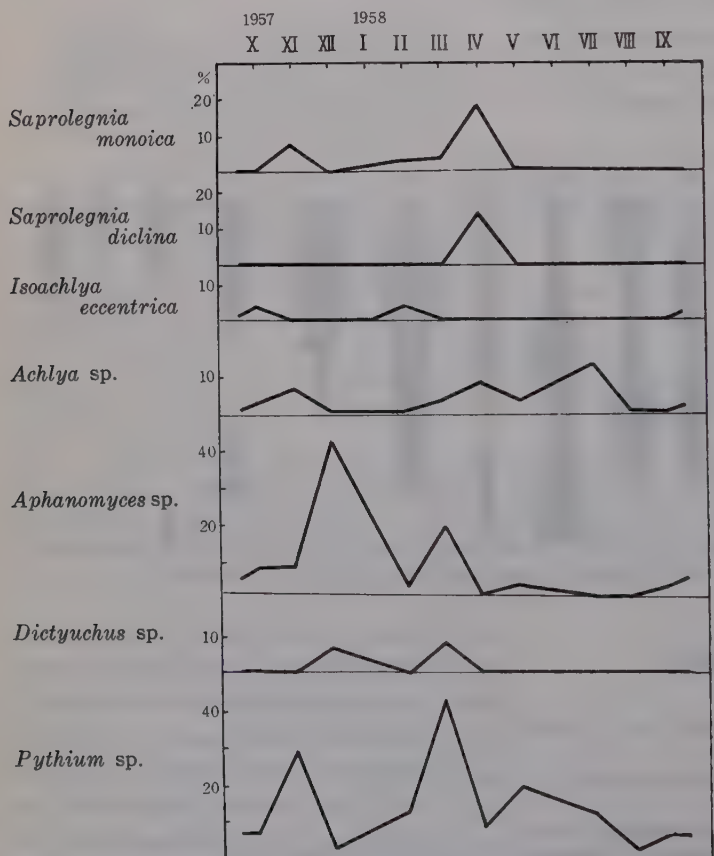
つの分布型が存在する．一つは全層一様に分布する型で循環期に見られ，他の一つはある限られた層にだけ見られる型で，停滞期に現われる．

このように水生菌類の遊走子の垂直分布は湖水の循環，停滞と相関を有しているが，遊走子は2本の鞭毛をもち，自由に遊泳できるので，環境の最適な層に集まることも考えられる．諸要因のうち，溶存酸素に対して感受性が大きいことがしばしば指摘されている[6,7]．著者も遊走子が懸滴水中で酸素の多い表層に集まることを観察している．震生湖の溶存酸素量の垂直分布は第3図に示すように，12月から4月までは酸素は表層から深層まで存在するが，湖水が停滞する5月から11月までは深層が無酸素状態になっている．遊走子の分布は酸素の溶存している層だけに限られ，酸素の垂直分布との相関が見られる．



第3図　溶存酸素の垂直分布

遊走子の一様な分布から成層的な分布に移行する場合，しばしば遊走子の消失が中層に現われる（第2図）．この時期の表層と深層では遊走子の種類が異なり，表層では *Saprolegnia* や *Aphanomyces* が，湖底直上では必ず *Pythium* が優占種である．



第4図　水生菌類の種類による季節的変化

B．種類の季節的ならびに垂直的分布

水生菌類の季節的消長を理解するために，これを種別に観察した結果を第4図に示した．震生湖から得られた種類は7種で，種数は3月，4月，11月に多い．出現頻度も春季と秋季に高く，この傾向は *Achlya* sp. を除いた全種類に共通であった．一年を通して見られる種類は *Aphanomyces* sp. と *Pythium* sp. の2種で，他の種類の出現する期間はきわめて短かい．

Saprolegnia monoica と *S. diclina* は春季と秋季にだけ見られたが，中沼[8]や占春池[9]でも同様な傾向が観察され，両種とも比較的水温の低い時期に繁殖が良いようである．*Dictyuchus* sp. の出現頻度は低く，秋から初春にかけて出現した．また *Achlya* sp. は比較的水温の高い時期

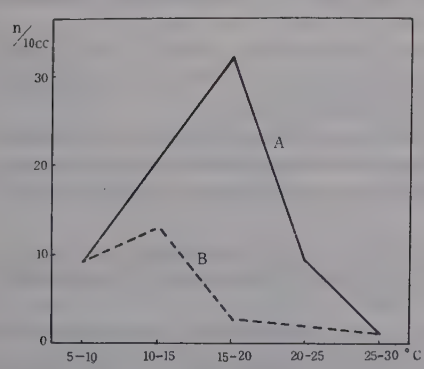
に多く，*Aphanomyces* sp. は水温の低下した冬季に最大に達する．*Pythium* sp. は1年を通じて常に分離されたが，なかでも春と秋の両季に出現頻度が最大となる．Waterhouse[4]の英国の川で *Pythium* sp. が1年を通じて出現するという報告は，本観察結果とよく一致している．

震生湖の1年を通じて支配的な水生菌類は *Pythium* sp. と *Aphanomyces* sp. で，*Achlya* sp. がこれに次ぐ．なお春季と秋季にまれに見られた *Isoachlya eccentrica* は本邦未記録の種であることを付記しておく．

つぎに各種類の垂直分布を見ると，*Aphanomyces* sp. の垂直分布は季節によって著しい変化を示す．特に注目すべきことは水温，溶存酸素が各層一様である循環期における分布である．この時期には *Aphanomyces* sp. は表層から水底まで分布するが，特に湖底に多い．この原因は *Aphanomyces* sp. の遊走子が遊走子嚢から出ると，遊走子嚢の頂端の孔辺に球形の塊を作って休止するので，水温の低下した冬季には第2遊泳期に入ることなく長時間この状態にとどまり，この遊走子塊が湖底に沈むので深層に多いものと思われる．*Pythium* sp. は停滞期には表層にだけ分布するが，循環期には表層から深層まで一様な分布を示す．

水生菌類の遊走子形成と二三の要因

水生菌類の消長を左右する要因として，水温，溶存酸素，栄養塩類などが考えられるが，遊走子形成に直接影響をおよぼす水温が最も注目される．震生湖の表面水温と遊走子数との関係は第5図のようである．この場合，冬季から夏季へ向かって水温の上昇にともなう遊走子数の変化を春カーブ，夏季から冬季に向かうのを秋カーブとする．



第5図 水温と水生菌類の遊走子数との関係
A: 春カーブ B: 秋カーブ

同じ水温においても，春カーブと秋カーブとで生産される遊走子の量が異なることは注目に値する．春カーブの最適温値は 15〜20°，秋カーブの最適温値は 10〜15° で低い．また両カーブの遊走子数を比較すると，各温度域で春カーブの方がはるかに高い．このような差異は水温以外の要因，特に水棲細菌類の増減が関係しているものと思われる．

湖沼の水棲細菌類の季節的消長に関しては，研究者によって結果は必ずしも一致していないが，Bere[10] は Wisconsin の湖沼で夏季に最大数を測定しており，他の多くの研究者によっても確かめられている．著者は夏季に細菌類が増加し，木の枝に発生している水生菌類の菌糸が細菌類の集落中にうずまってしまうことを観察し，Lund[1] も同様な事実を観察している．それゆえ細菌数の増加は水生菌類の活動力を弱め，その結果遊走子の形成が阻害されるものと思われる．

第1表 水温と各種類の出現頻度との関係(%)

水 温 °C	5〜10	10〜15	15〜20	20〜25	25〜30
Saprolegnia monoica	2	6	9	—	—
Aphanomyces sp.	12	27	4	2	2
Pythium sp.	28	14	8	14	6

つぎに震生湖に見られた主要な水生菌類の出現頻度と水温との関係を第1表に示した．*Saprolegnia monoica* は水温が 10〜20° の間に多く，水温が 20° 以上になった場合には全く見られない．Cotner[6] によれば，*S. monoica* var. *glomerata* の遊走子形成の最適温度は 26° であるというが，この値は著者の観測結果と著しく異なる．一般に *Saprolegnia* 属の菌糸は *Achlya* 属や *Dictyuchus* 属の菌糸にくらべて弱く，着生の細菌類の増殖による影響をより強く受けるものと思われる．*Aphanomyces* sp. の最適温度は 5〜15° で，それ以上に水温が上昇すると遊走子の形成が起こらない．*Aphanomyces euteiches* については Jones & Drechsler によれば，遊走子形成は 8〜35° の間で起こるという[11]．また Cotner は遊走子形成の最適温度は 26〜28° であるという[6]．震生湖での周年観察によれば *Aphanomyces* sp. は常に水温の低い時期に見られ，水温が上昇す

ると出現しなくなり，Cotner 等の研究結果と一致しない．一般に湖沼においては，水生菌類の遊走子形成は実験室での温度よりはるかに低い温度が良好なようである．*Pythium* sp. は 5～30° までの間で常に見られるが，特に春季と秋季に多い．

摘　　要

神奈川県の大秦野町にある震生湖において 1957年10月から翌年9月までの1年間，水生菌類の遊走子の季節的消長を観察し，つぎのような結果を得た．

1. 水生菌類の遊走子数は春季と秋季の2個の最大出現期と，夏季と冬季の2個の最小出現期が存在した．

2. 水生菌類の遊走子の垂直分布は季節による変化が著しく，二つの分布型が存在した．一つは全層一様に分布する型で，循環期に現われ，他の一つは遊走子が表層だけに分布するもので，夏季停滞期に見られる．

3. 水生菌類は7種より成り，*Saprolegnia monoica*, *S. diclina*, *Isoachlya eccentrica*, *Dictyuchus* sp. は水温の低い秋季から春季にかけて見られ，*Aphanomyces* sp. は冬季に多く，水温の高い夏季には見られない．*Pythium* sp. は1年を通じて出現したが，春季と秋季の水温の良好な時期に特に多い．*Achlya* sp. は水温の高い夏季に多い

4. 種類によって垂直分布は季節ごとに異なる．たとえば *Aphanomyces* sp. は停滞期には表層にだけ分布するが，循環期には底層に多い．*Pythium* sp. は停滞期には表層に，循環期には各層とも一様に分布する．

5. 水生菌類の遊走子数一温度曲線から，湖沼での遊走子形成は水温以外の要因，特に水棲細菌類の増減が関係するものと推論される．

終りにこの研究を御指導下さった東京教育大学の印東弘玄，伊藤洋両教授ならびに市村俊英講師に深謝する．また御鞭撻を賜わった東京理科大学の辰野高司教授に謝意を表する．

文　　献

1) Lund, A., D. Kgl. Danske Vidensk. Selsk. Skrifter, Naturv. og Math. Afd., 9 Raekke, **6**: 1 (1934). 2) Forbes, E. J., Mem. Proc. Manchester Lit. & Phil. Soc. **79**: 1 (1935). 3) Weston, W. H., Sym. Hydrobiol., Univ. Wiss. Press. 129 (1941). 4) Waterhouse, G. M., Trans. Brit. mycol. Soc. **25**: 315 (1942). 5) 鈴木静夫，陸水学雑誌 **21**: 17 (1960). 6) Cotner, F. B., Amer. Jour. Bot. **17**: 511 (1930). 7) Salvin, S. B., Mycologia **33**: 529 (1941). 8) 鈴木静夫，日生態会誌 **10**: 215 (1960). 9) ——，陸水学雑誌（投稿中）. 10) Bere, R., Int. Rev. Hydrobiol. Hydrogr. **29**: 248 (1933). 11) Jones, F. R., and Drechsler, C., Jour. Agr. Res. **30**: 293 (1925).

Summary

1) The seasonal variation in the amount of the zoospore of aquatic Phycomycetes was studied from October, 1957 to September, 1958 in Lake Shinseiko, Kanagawa Prefecture. The number of the zoospore increased both in spring and autumn, and decreased in summer and winter.

2) The vertical distribution of the zoospore varied with different seasons. There were two main types, i. e. the homogeneous distribution and the stratum one. The former appeared during the circulation period and the latter during the stagnation period.

3) Seven species were obtained from the lake. *Saprolegnia monoica*, *S. diclina*, *Isoachlya ecocentrica*, *Dictyuchus* sp. and *Aphanomyces* sp. were seen from autumn to early spring, and *Achlya* sp. was found mostly in summer. *Pythium* sp. was obtained through the year with the maximum in spring and autumn.

4) *Aphanomyces* sp. and *Pythium* sp. were distributed only in the surface layer during the stagnation period. During the circulation period, however, *Aphanomyces* sp. was distributed mostly in the bottom layer.

5) The zoospore production may be influenced not only by the water temperature, but also by the multiplication of periphytic bacteria. The optimum temperature for the zoospore formation in some species was as follows.

Saprolegnia monoica 10～20°, *Aphanomyces* sp. 5～15°, *Pythium* sp. 5～30°

Bot. Mag. Tokyo **73**: 487–493 (December 25, 1960)

双子葉類側生前葉の着生方向に関する研究
第1報 概　　論

熊　沢　正　夫*

Masao KUMAZAWA*: Analytical Studies on the Anodic and Cathodic Positions of Prophylls in Some Dicotyledonous Plants I. Introductory Remarks

1960年4月15日受付

序　　言

単子葉類では側枝第1葉，すなわち前葉 (Prophyll) が向軸側に着生する場合を原則とするに対し，双子葉類では少数の例外を除いて側生することは周知のとおりである．ら旋葉序を示す双子葉類では，この側生する第1前葉** (Prophyll α) の着生方向は主軸の基本ら旋を基準にすれば，それの上昇する方向 (anodic side) か，またはその反対方向 (cathodic side) かの2型に区分できる．前者の着生型式を進向型 (anodic type)，後者を退向型 (cathodic type) と呼ぶことにする (Fig. 1).

Hirmer[1]およびその一派は相次ぐ葉器が極限開度を示して逐次形成されることを強く主張するが，これは基本ら旋が葉器形成の内的傾向の反映像として実在することを意味する．それに反し，古来から現在にわたり，多くの人々は種々の立場から空間要素その他の内外要因により，そのつど個々の葉器分化の座が支配されると考え，また Plantefol[2] およびその一派は植物の内的要因により少数の斜列線 (Hélices foliaires) にしたがって葉器が形成されると考えている．これらの説では，基本ら旋は器官形成要因の反映像ではなく，単にわれわれの見掛け上の仮想線に過ぎないことになる．この点の当否は別として，現実に1本の基本ら旋を想定できる場合に，これの上昇方向を前葉着生方向記載の基準に便宜的に採用することは，観察結果の取り扱い方法を大いに単純化しうる．また一方その結果は，あるいは場合によって，基本ら旋そのものの存否，本質などに対しても，なんらかの示唆を与えるかもしれない．

著者は以前から，折にふれ木本を含む種々の植物の茎の一部を採り，その上の各側枝における前葉の着生方向を注目していたが，その結果は，1) 各前葉はいずれも大体に進向型である場合，2) 大体に退向型である場合，3) 両型が混在し，その間に特別の傾向の見られない場合とに一応区分できるとも見られたが，しかし同一種でもあまり判然としていない場合も多かった．また Troll[3] は *Lupinus polyphyllus* などの前葉はすべて退向型のように記しているが，該種について著者自身が見たところでは，必らずしもそのようではなかった．このような次第で，この問題はそれ以上追究しなかった．しかしその後，草丈 2 m に近いオオアレチノギク (*Erigeron sumatrensis* Retz.) の1個の直立茎を折取って，基部の側枝から順次上部の側枝にわたり，それぞれの前葉着生方向を観察した結果，下部側枝ではほとんどすべて退向型であるに反し，高所の側枝ではほとんどすべて進向型であり，中位の側枝では両者が混在していることを知った．この傾向はこの1個体に限らず，また近似種のヒメムカショモギ (*Erigeron canadensis* L.) についても同様であることを知るとともに，茎の任意の一部の所見が無意味なことをさとった．

これ以来，各個体について主茎の第1葉腋の側枝から，頂端部近くの側枝に至るまで，順を追うて各前葉の着生方向を調査し，同様の所見を多数の個体について集計して検討することに着手した．この種の詳細な研究は従来全く行なわれていない．したが

---

* Biological Laboratory, Department of General Education, Nagoya University, Nagoya, Japan. 名古屋大学教養部生物学教室

** 双子葉類側枝第2葉も普通前葉 (Prophyll β) と呼ばれるが，本文で単に前葉と記す場合には側枝第1葉のみをさすことにする．

って前葉着生の進向，退向の2型が1個体上において，いかなる変化の様式を示すか，また異種属間におけるこの様式の異同を明らかにすることが，以下続報する本研究の第一目的である．さらにこの知見を基礎として前葉着生方向決定の要因を分析し，ひいては一般葉序決定機構に対して，既往の研究者と異なる立脚点からなんらかの知見をうることを終局の目標とする．

葉序がいかなる機構のもとに決定されるかは，古来からの難題であって，従来主として二方向からの業績が蓄積されてきた．その一は葉序の規則性の観察に立脚して，この規則性の由来する要因を推論するもので，前世紀の Hofmeister[4]), Schwendener[5]) 以来の多くの葉序論はこれに属する．他の一は茎端に実験的手術を加え，葉序の変更を誘起せしめ，葉序の決定要因を分析追究するもので，Snow and Snow[6]) を先駆とし，その後両名以外による実験的報告も近年増しつつある．

葉序の変更は開度の変化の形で認識されやすいが，この開度変化は量的であり，これの測定が必らずしも正確を期しがたいのみならず，その結果から葉序変更の機構を考察するに際して，統計的処理が容易でない．それに反し，本題で取り扱う双子葉類前葉の着生方向は，原則として進向，退向の相反する明白な2型に限定されているので，個々の判定が明快であるし，その所見からの考察も簡単であることが特筆に値する利点である．このような前葉着生位置に関する所見は，2列互生 (distichous) 葉序の場合を除いて，従来ほとんど報告されていない．

双子葉類の横斜する側枝上の葉が2列互生する場合，その各腋芽の前葉はいずれも背地側に着生し，一種の背腹性を示す種類の多いことは従来良く知られているが，著者はイソノキ (*Frangula crenata* Miq.) などの4列互生葉序（前川のコクサギ型葉序と呼ぶもの）の側枝でも，前葉は必らず背地側に着生することを見ている．また主軸上の葉が2列互生する豆科植物では，直立性でありながら，第1次側枝前葉着生方向には原則として背腹性が顕著である．また *Vicia*, *Pisum* などの主軸上の各葉腋の主芽は，蓋葉 (subtending leaf) の正中線から交互に左および右側に偏位していて，この点で一種の背腹性を示すが，Dormer[7]) は主茎の基部附近の節における主芽が，その交互性を乱しているのに着目し，主芽着生位置の2型の決定要因分析を試みている．これは材料植物の葉序が著者の取り扱う場合と異なるだけで，着目点は著者と同じである．なんとなれば，Dormer は主芽の偏在方向のみを対照としているが，実は主芽が左側方に偏在する場合には，その前葉も必らずその左側に着生し，両者間に不可分の関連があるからである．

木原およびその協同研究者[8-14])が小麦の小穂の左右性として，主として遺伝学的見地から取り扱っている問題も，前葉着生方向の背腹性と，その乱れを対照とするものと見ることができる．なんとなれば小穂は穂梗の第1次側枝であり，小穂の第1苞穎は第1次側枝の側生第1前葉に相当し，第1小花は必らずこの第1苞穎と同側に着生するから，結局，前の場合と同じく前葉着生方向の問題となる．ただし2列互生葉序の場合には，方向の定まった基本ら旋を想定しえないので，これを基準として前葉着生方向を簡単に表現して取り扱うことができない．

材料および方法

材料植物としてなるべく次の条件に適合する種類を求めた．

1. ら旋葉序を示すもの．
2. 摘心することなくとも，子葉腋および主茎基部の節から高所の節に至るまで，もれなく順次腋芽が発生するもの．
3. 単軸分枝で直立茎を生じ，その頂端が花または花序に終るもの．
4. 少なからぬ数の節を有し，生長の早いもの．

その結果，材料は1年生または2年生の野生または栽培植物に限られることになった．材料植物はまれには自生のものを利用もしたが，ほとんどの場合，このために播種し，各個体をなるべく均等に管理し，適期に1個体ごとにまず基本ら旋の方向を確認して後，子葉・主軸上第1葉・第2葉・第3葉以下それぞれの腋芽の前葉着生方向を逐次調査する．同様のことを多数個体について行ない，これを集計して，主軸第N葉腋の側枝前葉（第N節位の前葉と略称する）が退向型を示す場合が，第N節位前葉全数中いかほどの頻度になるかをパーセントで表示する．

基本ら旋の方向は植物を真上からみおろして，第1葉・第2葉以下が時計廻りの場合と逆時計廻りの

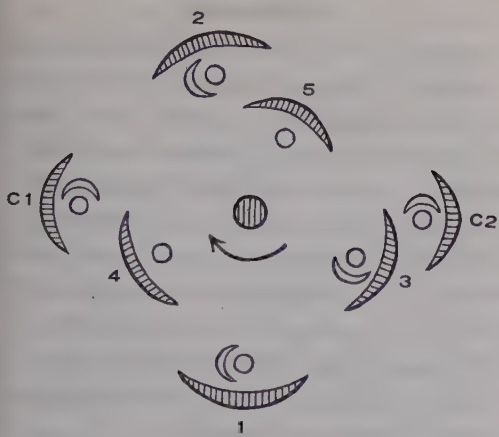


Fig. 1. Seedling phyllotaxis of clockwise spiral system. C1, C2: opposite cotyledons. The prophylls of branches subtended by C1, 1 and 3 are of anodic type, those subtended by C2 and 2, of cathodic type.

場合とがあるが，本研究の植物では，いずれも基本ら旋の方向は一応第2葉の位置で識別される．しかし子葉は対生するし，第1葉・第2葉間の開度も180° に近いから，第3葉以後の着生位置から基本ら旋の方向を判定し，つぎに第4葉・第3葉・第2葉の順に基本ら旋を退行方向に追跡する時，Fig. 1 のごとく第1葉のつぎに位置する子葉を第2子葉 C2，これに対生する他方の子葉を第1子葉 C1 と名づけ，第1子葉を基本ら旋の出発点と仮定して，それぞれの節位の前葉着生方向を進向型・退向型のいずれかに表現する．たとえば図中 C1，1，3 をそれぞれ蓋葉とする側枝前葉は進向型，C2 および 2 のそれは退向型である．

主軸の基本ら旋の時計廻り，または逆時計廻りを示す個体の頻度は，いずれの種においても有意義の差はないので，退向型前葉頻度算出上では，ら旋の方向の別は無視して合算した．基本ら旋の方向が1単軸上の途中で逆転したり，葉序の規則性が混乱して，一定のら旋を想定できないような個体もまれには見られた．後者のごとき不規則葉序は特にホウセンカに比較的頻度多く現われたが，それらの個体は一応集計から除外した．

一個体の植物について，主軸第1葉腋の側枝から主軸伸長後の高所の側枝にわたり，それぞれの前葉着生位置を一時に記録することは一般には不可能である．なんとなれば主軸高所に腋芽が発達する時期には，主軸基部附近では葉がすでに失われて節位が不明となるのみならず，腋芽も枯死したり，あるいは過大に生長して前葉着生型を確認できないからである．したがって多数の個体を栽培し，その一部を適期に抜き取って下位の側枝前葉を調査し，残りの苗はさらに発育して高所の腋芽が発生したときに，改めてその部位附近の前葉位置を調査し，これを前者のデーターと連絡させる．無論後者の材料を用いて高所から発生する側枝の前葉を調査する時，主軸基部附近の節位が不明になっていても，そこが主軸の第何節位であるかが判定できるように，あらかじめなんらかの工夫をしておかねばならない．

以上は第1次側枝前葉着生型を，主軸上の基本ら旋の進行方向を基準として取り扱う場合であるが，第2次側枝の前葉についても同様の方法で，第1次側枝の基本ら旋の方向を基準にした．側枝の基本ら旋の方向が第2葉の座によってすでに決定するか否か問題である．しかし観察の実際上では，第3葉の

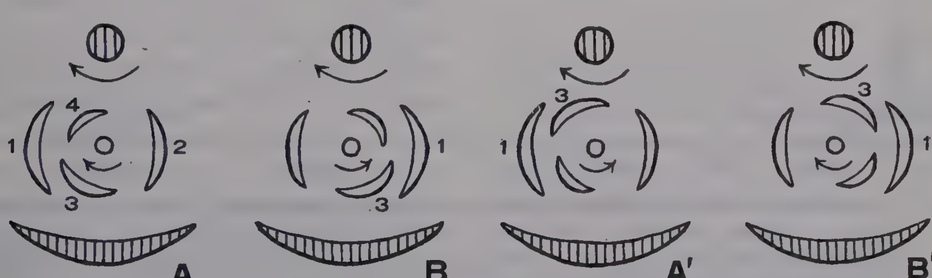


Fig. 2. Phyllotaxis of the proximal part of the axillary branch, showing the direction of genetic spiral (short arrow). In all figures the genetic spiral on the mother axis is clockwise (long arrow). A, A′: prophyll (1) anodic. B, B′: prophyll cathodic. A, B: third leaf (3) abaxial. A′, B′: third leaf adaxial.

位置によって識別されるが，主軸と側枝との基本ら旋の進行方向は必らずしも一致しない．側枝第3葉は双子葉類では背軸側に位置することが普通であるが (Fig. 2, A, B)，向軸側の場合もある (Fig. 2, A′, B′)．図で明らかなように，第3葉が背軸側にあるときに進向型前葉で始まる側枝の基本ら旋は，その母軸の基本ら線とその進行方向が同一であるが (Fig. 2, A)，退向型前葉で始まる側枝の基本ら旋の方向は，母軸のそれと不一致であり (Fig. 2, B)，第3葉が向軸側に位置するときには (Fig. 2, A′, B′)，この関係が逆になる．

第2次側枝前葉の退向型頻度を算出する場合，節数の10数個におよぶ長大な第1次側枝のみを材料として選び，その結果を集計したが，若干の種類では，生長後における節数の多いもの少ないものを節数に応じて区分し，別々に集計してその差異を検討した．

第3次以高の側枝上の前葉着生型の変化は，予備的所見から第2次側枝上の前葉の場合と相違ないように思われたので，現在までには詳細な研究を行なっていない．

一集計に用いた材料数は1000個体以上に及んだ種類もあるが，少なくとも200個体以上を取り扱うよう努めた．

なおある種では，播種期を変えた材料同士の比較，早生品種と晩生品種との比較，あるいは自然状態下のものと日照時間を変更したものとの比較なども行なった．

退向型頻度曲線

オオアレチノギク主軸上の第1葉から頂端部に至るそれぞれの葉腋から発生する第1次側枝の前葉着生型を，多数の個体について，各節位の側枝（横座標．矢印は主軸上または第1次側枝上の最下位側枝を示す）ごとに集計して得た退向型の頻度（縦座標）を示したものが Fig. 3 太線であり，これを前葉退向型頻度曲線 (Frequency curve of cathodic prophylls) と呼ぶことにする．ただし図は多少模式的に表現してある．Fig. 4, B の太線はそれと同じものであるが，さらに一層概観的に単純化して図示してある．このような概観的変化傾向を退向型頻度の一般変化 (General variation) と呼ぶ．

集計が大なるほど，各節位側枝間における頻度の鋸歯的変動は相殺されて，頻度曲線がなめらかとなることは一応想像できるが，Fig. 3 太線のごとく，茎の下部では各節位側枝によって退向型前葉の頻度にいちじるしい変動があり，集計を増大させても，その鋸歯的変化が相殺されない．この基部節位の側枝前葉に限る特異の頻度の変動を基部変化 (Basal variation) と名づけるが，これは基部に特有のなんらかの要因によって誘起される基部効果 (Basal effect) と見られる．なお前記 Fig. 3 太線の右端，すなわち主軸頂端部に近い節位からの側枝前葉の退向型頻度は，曲線全体の傾向から見ると急激な変化を示しているので，この部分を特に末端変化 (Terminal variation) と呼び，これも末端部特有の要因の反映した末端効果 (Terminal effect) と考えられる．

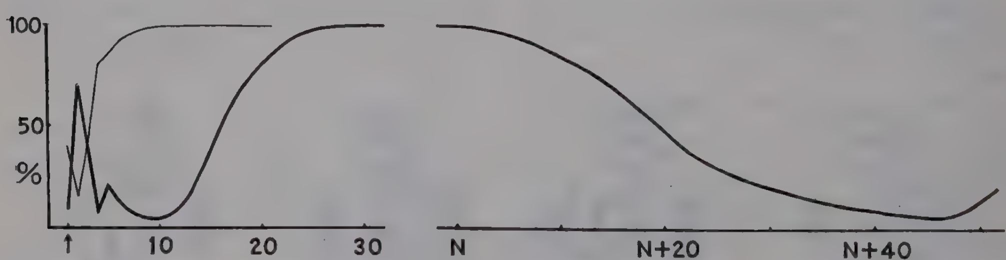


Fig. 3. Frequency curve of cathodic prophylls in *Erigeron sumatrensis*. Ordinate: percentage of cathodic prophylls. Abscissa: ordinal numbers of primary or secondary branches in the genetic sequence. The arrow indicates the lowest branch subtended by the first leaf of the main axis or of its primary branch axis. Thick line: frequency curve of cathodic prophylls of primary branches. Thin line: that of secondary branches accompanied with numerous leaves. This line extends in fact further rightwards nearly as the thick line.

Fig. 3 細線はオオアレチノギクの第2次側枝前葉の退向型頻度曲線であるが，右方へ伸びる曲線は大体に太線と同一傾向をとるから，図示を省略してある．これを見ると，基部変化は第1次側枝前葉の場合と若干の差があるが，この部を除く一般変化の様式は第1次側枝前葉の場合と異ならない．ただしこのことは第1次側枝が主軸と同様，長大に良く発達して節数もきわめて多い場合における第2次側枝前葉着生型に限ってあてはまるのであって，節数の少ない短小な第1次側枝上における第2次側枝前葉退向型頻度曲線では，末端効果が早く反映するため，前記の曲線と完全には一致しないことがあるが，これについては後にさらに述べる．

退向型頻度の一般変化の類型

著者が現在までに研究した1年生および2年生植物は約 10 種にすぎないが，それらの第1次側枝前葉退向型頻度の一般変化の様式 (Fig. 4, 太線*) をしいて大別すれば，つぎの3型とすることができよう．

A．基部変化を経過後，進向型に終始するもの――オオオナモミ (*Xanthium canadense* Mill.) (Fig. 4, A)

B．基部変化を経過後，かなり長く退向型を維持した上で，進向型に近づくもの――オオアレチノギク (*Erigeron sumatrensis* Retz.), (Fig. 4, B)

C．基部変化経過後，早晩退向型になるもの．これをさらに細分すれば 1) 直ちに退向型となるもの――ホウセンカ (*Impatiens Balsamina* L.) の早生品種 (Fig. 4, C1) 2) 途中で進向型を軽微に現わすもの――ホウセンカ晩生品種 (Fig. 4, C2) 3) 途中明白に進向型の時期の現われるもの――ホウキグサ (*Kochia Scoparia* Schrad.,) (Fig. 4, C3).

Fig. 4 細線は良く発育した第1次側枝上の第2次側枝前葉退向型頻度の一般変化であるが，線の右端は太線のそれと大体に重複するので途中で省略してある．すなわち上のいずれの型にあっても，基部変化を除く第2次側枝前葉の一般変化の様式は，第1次側枝前葉の示す曲線の短縮化または単純化，換言すれば個体発育の後期を示す特性が第1次側枝上では比較的下部の節位の側枝において前葉着生型の上に反映しているといえる．すなわち図で明らかなごとく，ホウキグサ，ホウセンカ，オオ

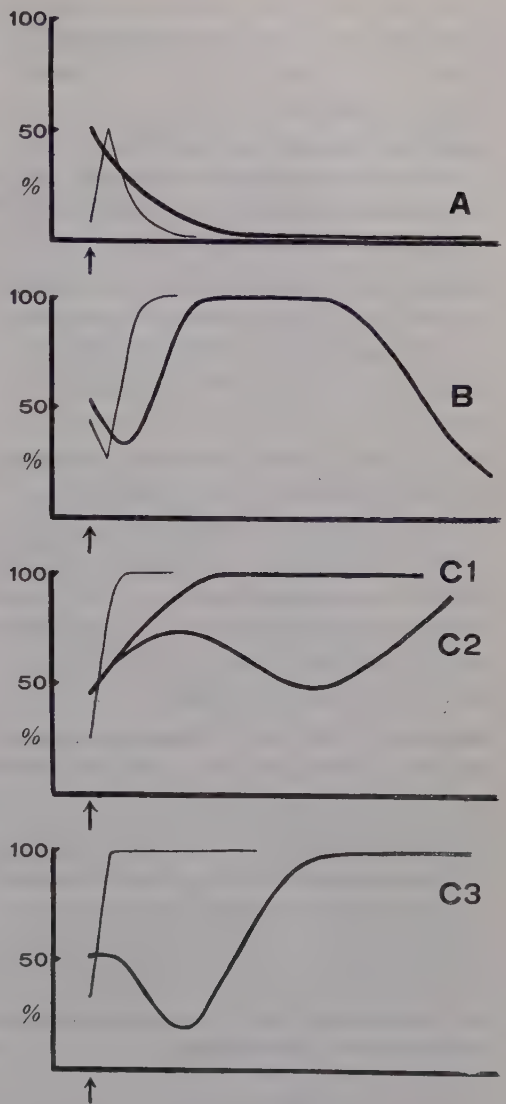


Fig. 4. Frequency curves of cathodic prophylls, diagrammatically simplified. A: *Xanthium canadense*. B: *Erigeron sumatrensis*. C1: *Impatiens Balsamina*. An early variety. C2: the same species. A late variety. C3: *Kochia Scoparia*. Explanation of graphs as in Fig. 3.

* 基部変化および末端変化は一局部の特性であるから，図の曲線には具体的には表現させてない．

アレチノギクでは，主軸にくらべて第1次側枝の節位の低い所で，すでに第2次側枝前葉の退向型頻度が100%に近づいているし，オオオナモミでは逆にすみやかにその頻度が低下している．この所見および続報で詳述する発育の良否の材料間，あるいは早生・晩生品種間の比較の所見などから推せば，第1次側枝前葉着生方向のC1型曲線はC2型・C3型の簡略型であり，さらにまた，これらのすべてはB型曲線の右半分が省略された一種の短縮型と考えることができる．たとえばFig. 4, C2の曲線を示すホウセンカ晩生品種に対し，播種期をおくらせて生長期を短縮すると，途中の曲線の谷が浅くなりC1曲線に近づくし，オオアレチノギクの節数の少ない弱小な主軸における第1次側枝前葉退向型頻度曲線は，図中のB曲線が左右に圧縮された形よりも，むしろ曲線の右端の部分が省略された形を示し，極端な場合には退向型頻度が100%からわずか低下したとき，すでに主軸は末端となる．

従来研究した種類はこれらA—Cのいずれかの型におおよそ合致する．すなわちミズナ(*Brassica Rapa* L. var. *laciniifolia* Kitam.)，ナタネ(*B. Napus* L.)，クロタネソウ(*Nigella damascena* L.)は大体にオオアレチノギクと同様B型類似の曲線を示すが，それより山が低く谷が浅い．これらの種類は普通は越年性であるが，春まきの材料でも曲線の基本的様式は同一である．イヌビユ(*Amaranthus ascendens* Loisel.)はホウキグサ，ホウセンカとともに1年生であるが，退向型頻度曲線もホウセンカ早生品種と同一の様式，すなわちC1型を示す．オオオナモミに見るごとき型式は筆者が取り扱った種類中には他にその例を見ない．

基部変化

前葉退向型頻度の一般変化が本来いかなる機構のもとに規定されるかは興味ある問題であり，目下Plastochroneの個体発生中の変化の所見などから検討しつつある．したがってこの点については後の機会に譲るが，著者の第一の関心は基部変化にある．一般変化には個体の発育の良否，生長期の長短などにより，ある程度の差異が見られるに対して，第1次側枝前葉，第2次側枝前葉ともに，それぞれの基部変化の様式には著しい差異が見られないからである．すなわち，オオアレチノギクについていえば，主軸第2節位および第5節位の側枝前葉の退向型頻度の値そのものは，統計材料の集団により若干の変動があるが，それらの頻度がその上下の節位の側枝前葉のそれより明らかに高い値を示す現象(Fig. 3)は外因などにより容易に変更されない．さらにこれと同型式の鋸歯的基部変化が，他の種属にも等しく見られることに注目しなければならない．この2個の現象は第2次側枝前葉の場合の基部変化にも適用される．この事実は主軸基部および側枝基部における葉序が，他の部位と明白に相違することの反映と考えられる．オオオナモミの第1次側枝第3葉は向軸側に着生する場合と背軸側に着生する場合とがあるが，両者の間で第2次側枝前葉の退向型頻度の基部変化はかなり明白な差異のあることを見いだしている．このことはさらに一層前記の前葉着生型の基部変化が母軸の特殊の葉序の反映結果であることを暗示するものであり，基部変化こそ前葉着生型の決定機構分析の重要な手掛かりとなると信ぜられるので，続報でさらに詳細検討する．

末端変化

Fig. 3の太線右端の小部分を末端変化の例として前に指摘したが，末端効果はオオアレチノギクの場合でも，退向型頻度の値を少し向上させるばかりとは限らない．すなわち，相次ぐ節位の側枝前葉の退向型頻度が大きい部位に末端効果が作用すると，その頻度は一般にかえって低下する．オオアレチノギクの第1次側枝では，頂端から数えて数個以内の節位の第2次側枝前葉着生型の頻度に，末端効果が反映すると思われるが，節数の特に少ない短小な第1次側枝においては，その節数の差により第N節位から出る第2次側枝の前葉退向型頻度はある場合には異常に高く，他の場合には異常に低いことがあり，その結果，節数の多い長大な第1次側枝に見られる末端変化とは異なる様式を示す場合がある．これはおそらく節数の少ない第1次側枝では，末端効果と基部効果とが重複する結果と考えられる．

末端変化の所見は数種についてさらに具体的に続報するつもりである．これは基部変化とともに前葉着生型の決定機構分析の手掛かりを与えるものと思われるからであるが，実は末端効果の要因は基部効果の場合より複雑であるらしく，現在のところ著者はまだそれに関しては追究の緒についていない．

本研究の費用の一部は文部省科学研究交付金による.

文 献

1) Hirmer, M., Zur Lösung des Problems der Blattstellung. Jena (1922). 2) Plantefol, L., Ann. Sci. Nat., Bot. **11** : 153 (1946). 3) Troll, W., Vergleichende Morphologie der höheren Pflanzen. **1** (**2**) : 334 (1936). 4) Hofmeister, W., Allgemeine Morphologie der Gewächse. Leipzig. (1868). 5) Schwendener, S., Mechanische Theorie der Blattstellung. Leipzig. (1878). 6) Snow, M. and Snow, R., Phil. Trans. Roy. Soc. London, B. **221** : 1 (1931). 7) Dormer, K. J., Ann. Bot. N. S. **18** : 55 (1955). 8) Kihara, H., Kimura, M. and Ono, H., Proc. Jap. Acad. **27** : 678 (1951). 9) Suemoto, H., ibid. **29** : 362 (1953). 10) Kojima, K., ibid. **29** : 576 (1953). 11) Kojima, K., Suemoto, H., Ono, H. and Sueoka, N., ibid. **30** : 214 (1954). 12) Ono, H., Sueoka, N., Suemoto, H. and Kojima, K., ibid. **30** : 221 (1954). 13) Suemoto, H. and Kojima, K., Wheat Inf. Service **2** : 10 (1955). 14) Sueoka, N. and Murai, T., Proc. Jap. Acad. **32** : 191 (1956).

Summary

In this paper, purposes, methods and materials were described and a part of the results obtained in some species of annual and biennial dicotyledonous plants was preliminarily reported. Special studies in detail will appear in the future papers of this series.

The primary and secondary branches were observed in genetic sequence from the lowest one towards the upper along the respective mother axis. After the observation on hundreds of branches, the cathodic prophylls on the branches of the same genetic number were added together. The results thus obtained in each species were expressed by the frequency curve of cathodic prophylls of its own.

In the case of the primary branch, the frequency curves may be divided by their trend into three types, i.e. type A (*Xanthium canadense* Mill., Fig. 4, A), type B (*Erigeron sumatrensis* Retz., Fig. 4, B; *Nigella damascena* L., *Brassica Napus* L., *Brassica Rapa* L. var. *laciniifolia* Kitam.) and type C (*Impatiens Balsamina* L. Fig. 4, C1, C2; *Kochia Scoparia* Schrad., Fig. 4, C3; *Amaranthus ascendens* Loisel.). In the case of the well-developed secondary branch, the frequency curve of cathodic prophylls is fundamentally similar in each species to that of the primary branch, but it is somewhat simpler.

In every species the cathodic prophylls on the branches situated at the basal and terminal parts of a main axis and of a primary branch axis show characteristic local variations of frequency, different from the general trend of the frequency curve. It was preliminarily interpreted that these basal and terminal variations have resulted from some factors such as the special phyllotaxis, the plastochrone change, etc., characteristic to the basal and terminal parts of an axis. Analytical studies on the basal and terminal effects will give some suggestions upon the mechanism which determines the anodic or cathodic positions of prophylls.

Bot. Mag. Tokyo **73** : 494-496 (December 25, 1960)

ヒロメの遊走子形成*

西林長朗**・猪野俊平**

Takeo NISHIBAYASHI** and Shumpei INOH**: The Formation of Zoospores in *Undaria undarioides* (Yendo) Okamura*

1960年4月30日受付

ヒロメは1903年に遠藤[1]によって発見され, *Hirome undarioides* Yendo として記述された. しかし, この植物はワカメによく似ていて, 葉に切れ込みがないことと, 成熟すると中肋部に子嚢群をつけることだけがワカメと異なっている. そこで岡村(1915)[2]はヒロメはワカメに近縁な種であると考え, ヒロメ属を廃してワカメ属に統合して, ヒロメを *Undaria undarioides* (Yendo) Okamura として記載した. ヒロメは本邦特産の植物であるが, わが国でもその分布が限られていて, 和歌山県の一部と, 志摩半島の南岸, 房州船形などに産するのみである. 近年, 瀬木・喜田(1957, '58)[3,4]はヒロメの遊走子を培養して配偶体の発生について観察を行ない, 雌雄配偶体の生卵, 造精器の成熟までの発生過程は, ワカメの配偶体の発生と比較して, ほとんど差異がないこと, さらに配偶体および芽胞体の発生は, 光線の強さによって著しく影響されることを報告している. しかしヒロメの核学的研究についてはいまだ全く報告がないので, 著者らはヒロメの遊走子形成の際の核学的観察を行なったところ, 新しい知見を得たのでその結果を報告する.

材料と方法

本研究に用いたヒロメ(*Undaria undarioides* (Yendo) Okamura)は, 1958年3月24日に和歌山県田辺湾で採集したものである. 採集した材料はまず酢酸オルセインを用いて遊走子嚢の成熟程度を確かめ, 葉の下方にできた胞子嚢群の適当な部分を切りとり, これを細かく切って阿部氏液[5]で固定した. 固定時間は15~20時間である. 固定後はパラフィン切片法により5~6 μ の切片を作り, 10% 過酸化水素水で約45時間漂白した後, ハイデンハイン氏鉄明礬ヘマトキシリンで染色して観察を行なった.

観察

ヒロメの胞子嚢群は葉の中央部の中肋の両側において, 表裏両面で同時に形成されはじめ, 葉の基部に向かって形成が進み, 後には葉の下方, 全面にわたって作られるようになる. 胞子嚢群をつけた部分は色が濃くなっている. 遠藤(1911)[6]によると, ヒロメはワカメと同じように, 柄の両側に波状に屈曲した胞子葉をつけるものがあるといわれている. しかし著者らが採集したヒロメには, このような胞子葉をもった個体は見られなかった.

胞子嚢群は生殖器官である遊走子嚢と, それを保護する役目をもつ側糸とからできている. それらの発生については著者ら(1960)[7]がすでに報告している. Fig. 1は下位細胞から切り出されて間もない遊走子母細胞(若い遊走子嚢細胞)を示す. この細胞には一つの静止核と数個の色素体が含まれ, 核の中には濃く染まる球形の仁と, 繊細な網目状の構造が観察される. 静止核の中, 約15% のものには二つの仁が存在する(Fig. 2). 核分裂の経過が進むにつれて, 核は大きくなるとともに網目状の構造が一層明瞭となってくる. その後, 濃く染色されるようになった染色糸はループ(loops)を作って, 核腔内の一隅に集まる. いわゆるシナプシス期(synapsis)である. 染色糸の幾つかのものは常に仁と密着している(Fig. 3). 核の一隅に集まった染色糸は核腔内いっぱいに拡がり, そこに濃く染められた染色糸の網目が形成される(Fig. 4). この時期がオープ

---

* 文部省科学研究費, 課題番号 407125
岡山大学理学部生物学教室植物形態学研究業績 No. 76
玉野臨海実験所業績 No. 69

** Department of Biology, Faculty of Science, Okayama University, Okayama, Japan. 岡山大学理学部生物学教室

ン・スピレム期 (open spireme) である．その後に染色糸は各所で肥厚してくる (Fig. 5)．染色糸の肥厚短縮は核内で一様に進まないので，一つの核の中で粒状の染色体になっているものもあれば，まだ染色糸の状態が残っているものもある (Fig. 6)．ディアキネシスの核は核分裂の経過の中で，その大きさが最大となり直径は約 6 μ に達するようになる．核内には約30個の X, O, V, II などの形をした二価染色体が散在している (Fig. 7)．このような二価染色体はさらに肥厚短縮をつづけて，小さい粒状の染色体となり中期に入っていく．仁はディアキネシスのはじめにはなお存在しているが，二価染色体が粒状の形になる頃には消失する．

中期では二価染色体は赤道板に整然とならび，核膜は不明瞭になる．その側面観では，染色体は中央でくびれて啞鈴状を呈している．紡錘体は非常に繊細であるけれども認められる (Fig. 9)．中心体および星状体は観察されない．極面観では小さい粒状の染色体が一平面上に配列しているのが観察されるが，核板は著しく収縮している．そのため中期の染色体の観察は，ディアキネシスのものよりもいくらか困難である． Fig. 8 は中期極面観像を示し，約 29 の染色体が数えられる．本植物の染色体は非常に小さいので，個々の染色体の形態的特徴を識別することはできない．紡錘体の軸の方向は遊走子嚢の長軸に平行か，または少し傾むいている場合が多く，直角な場合はまれである．中期像は多数観察されたが，ディアキネシスの像は中期のものにくらべるとわずかしか観察されなかった．後期では各二価染色体は一価染色体に分かたれて，規則正しく両極に向って動いていく (Fig. 10)．終期には核膜も仁も再び現われて 2 嬢核が形成される (Fig. 11)．

第一核分裂の完了後， 2 嬢核の間には隔膜が形成されることなく，直ちに第二分裂がはじまりその結果 4 核が作られる (Fig. 14)．第二分裂における遊走子嚢内の二つの核分裂はほとんど同時に行なわれる．これら 2 核の分裂の方向は相互に関係はない．二つの分裂像は平行な位置をとることが多いが，たがいに直角の位置を占める場合も観察された．第二分裂の中期，後期は正常に経過し，染色体は規則正しく両極に別れていく (Figs. 12, 13)．この時の紡錘体は第一分裂のと同じように繊細であり，また中心体も星状体も観察されない．

その後，引き続き 3 回の核分裂が行なわれて，遊走子嚢内には 32 の核が散在するようになる．これらの核分裂はすべて同時的に行なわれるが，分裂の方向は不規則である (Figs. 15, 17, 18)． 核分裂の回数を重ねるにしたがって，核および染色体はだんだん小さくなっていく．第四および第五分裂では紡錘体は不明瞭である． 8 核期までは繊細であるけれども核膜は観察されて，核の存在が確かめられる (Fig. 16)．しかし 16 核期および 32 核期では核膜は不明瞭となり，遊走子嚢内には濃く染められた仁が点々と認められるだけである．それゆえ，第五分裂終了後の 32 の遊離核を確認することはできなかったけれども，第五分裂より後の分裂は観察されないし，また第五分裂では 16 個のすべての核が同時に分裂しているので，一つの遊走子嚢内には 32 の遊離核が形成され，それ以後は核分裂を行なわないと思われる． 4 核期から 32 核期までの間に遊走子嚢は急速に成長する．第五分裂の終了後，遊走子嚢の頂端の膜は肥厚して粘液帽 (mucilage-cap) が作られる．32 の遊離核が作られた後は，おのおのの核を中心として遊走子が形成され，その結果一つの遊走子嚢内には 32 の遊走子が含まれている (Fig. 19)． 完熟した遊走子嚢の大きさは長さ 60〜86 μ，幅 8〜12 μ である．

考察および結論

以上の観察の結果，ヒロメの遊走子嚢内の第一核分裂で，シナプシス期およびいろいろな形をした二価染色体が核腔内に散在しているディアキネシスが確認されたので，遊走子嚢内の最初 2 回の核分裂が減数分裂である．ディアキネシスに約 30，第一分裂中期に約 29 の染色体が数えられた．それゆえ，ヒロメの半数染色体数は約 30 である．コンブ目植物の中で，これと同じ染色体数をもつものには，ヒロメのほかにスジメ (西林・猪野，1957)[8] およびワカメ (猪野・西林，印刷中) がある．ミツイシコンブ (西林・猪野，1956)[9] とスジメ[8]) では，オープン・スピレム期の後に染色糸が肥厚してくる頃から仁は消失しはじめ，ディアキネシスでは完全に消失しているが，ヒロメではワカメ (猪野・西林，印刷中) と同じように，ディアキネシス末期まで仁は残存している．このようにワカメ属に属するヒロメとワカメにおいて，仁の消失がおそいので，このこ

とはワカメ属 (*Undaria*) の特徴と思われる．第一分裂中期の像は多く観察されたが，ディアキネシスの像は中期像ほど多数は観察されなかった．このことから中期は比較的ゆっくりと経過するが，ディアキネシスは非常に速く経過すると判断される．ヒロメの核膜は繊細で，16 核期および 32 核期に核膜が不明瞭になることは，紡錘体が繊細であることとともに本種の特異な性質である．

減数分裂にひきつづいて 3 回の核分裂が行なわれて，遊走子嚢内には 32 の遊離核が形成される．その後，それらの核を中心にして遊走子が作られるので，一つの遊走子嚢内には 32 の遊走子が含まれている．ヒロメ以外に，***Alaria esculenta*** (Sauvageau, 1918)[10], ***Laminaria saccharina*** (Schreiber, 1930)[11], ***Pelagophycus porra*** (Herbst and Johnstone, 1937)[12], ***Eisenia arborea*** (Clare and Herbst, 1938)[13], マコンブ (阿部, 1939)[14], ワカメ (猪野・西林, 1954)[15], ミツイシコンブ (西林・猪野, 1956)[9], スジメ (西林・猪野, 1957)[8], チガイソ (籔, 1957)[16], オニコンブ (籔, 1958)[17] などコンブ目植物の多くのもので 32 の遊走子が形成される．

本研究を行なうに当り，材料の採集および実験に多くの便宜をお計り下さった，和歌山県水産試験場の所員の方々に厚くお礼申し上げます．

文　　献

1) Yendo, K., Bot. Mag. Tokyo **17**: 99 (1903). 2) Okamura, K., ibid. **29**: 266 (1915). 3) Segi, T., and Kida, W., Rep. Fac. Fish., Pref. Univ. Mie **2**: 517 (1957). 4) ——, and ——., ibid. **3**: 236 (1958). 5) Abe, K., Sci. Rep. Tohoku Imp. Univ., Biol. **8**: 259 (1933). 6) 遠藤吉三郎，海産植物学 (1911). 7) 西林長朗・猪野俊平，植雑 **73**: 75 (1960). 8) ——・——，同 **70**: 228 (1957). 9) ——・——，同 **69**: 501 (1956). 10) Sauvageau, C., Mém. Acad. Sci., Paris **56** (1918). 11) Schreiber, E., Planta **12**: 331 (1930). 12) Herbst, C. C., and Johnstone, G. R., Bot. Gaz. **99**: 339 (1937). 13) Clare, T. S., and Herbst, C. C., Amer. Jour. Bot. **25**: 494 (1938). 14) Abe, K., Sci. Rep. Tohoku Imp. Univ., Biol. **14**: 327 (1939). 15) Inoh, S., and Nishibayashi, T., Biol. Jour. Okayama Univ. **1**: 217 (1954). 16) 籔　　凞，北大水産研究彙報，**8**: 185 (1957). 17) 籔　　凞，藻類 **6**: 57 (1958).

Summary

1. At the first nuclear division in the zoosporangium of *Undaria undarioides* (Yendo) Okamura, synapsis stage and diakinesis are observed. Therefore, the first and second nuclear divisions in the zoosporangium are meiosis.

2. After meiosis, three successive mitoses take place to form 32 free nuclei. Consequently 32 haploid zoospores are contained in a zoosporangium.

3. The haploid chromosome number in the present species is about 30.

4. Both the centrosome and the aster are not observed. The spindle is delicate.

5. The nucleolus disappears at late diakinesis.

Plate I. Formation of zoospores in *Undaria undarioides* (Yendo) Okamura. All magnifications ca. × 2600.

Fig. 1, Resting stage. Fig. 2, The same stage, showing two nucleoli in the nuclear cavity. Fig. 3, Synapsis stage. Fig. 4, Open spireme stage. Figs. 5, 6, Early diakinesis. Fig. 7, Diakinesis, showing X–, O–, V–, II–shaped bivalent chromosomes. Fig. 8, Polar view of the metaphase. Fig. 9, Side view of the same stage. Fig. 10, Anaphase. Fig. 11, 2 daughter nuclei. Fig. 12, Metaphase of the second meiotic division.

T. Nishibayashi and S. Inoh: The Formation of Zoospores in *Undaria undarioides* (Yendo) Okamura.

Plate II

Plate II. Formation of zoospores in *Undaria undarioides* (Yendo) Okamura. All magnifications ca. × 2600.

Fig. 13, Anaphase of the second meiotic division. Fig. 14, 4 nucleate stage. Fig. 15, Metaphase of the third nuclear division. Fig. 16, 8 nucleate stage. Fig. 17, Metaphase of the fourth nuclear division. Fig. 18, Metaphase of the fifth nuclear division. Fig. 19, Zoospores in a zoosporangium.

T. Nishibayashi and S. Inoh: The Formation of Zoospores in *Undaria undarioides* (Yendo) Okamura.

Bot. Mag. Tokyo, **73**: 497 (December 25, 1960)

Short Communication

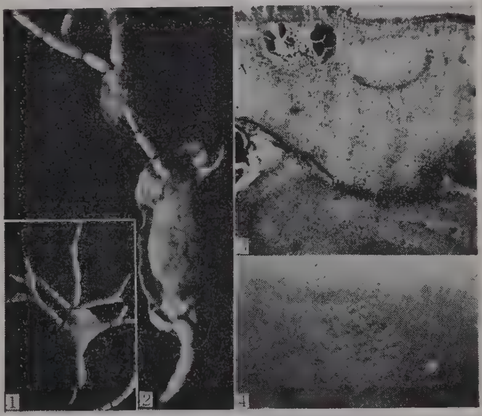
Jun Tokida and Tomitaro Masaki*: On the Occurrence in Japan of a Crustaceous Coralline, *Polyporolithon*

時田　郁*・正置富太郎*:　日本新産無節石灰藻 1 種について

Received October 24, 1960

In the course of the writers' studies on the Melobesioideae of Japan[1-3]), they have come across a small crustaceous coralline attached to the thalli of *Pachyarthron cretaceum* collected in Hokkaido at Muroran in March 1960 and at Shirikishinai in August 1960. After a thorough study it is concluded that the plant in question is identical with *Polyporolithon reclinatum* (Foslie) L. R. Mason in almost every respect as will soon be described in Part IV of the writers' work treating of the Melobesioideae of Japan. *Polyporolithon reclinatum*, which to date has been known only from the Pacific coast of North America, is now reported in this communication to be new to Japan. This is also a new addition to a list of the marine algae occurring on both sides of the North Pacific.

Polyporolithon was established in 1953 by Mason[4]). He stated, "*Polyporolithon* differs from *Lithothamnium* in its hemiparasitic habit and its characteristic mushroomlike growth form." A vertical section through the base of one of the writers' specimens shows that it penetrates into the tissue of the host (Fig. 3). In the present species both tetrasporangial and cystocarpic conceptacles have been described by Mason[4]), but male individuals remain unknown to date. The writers fortunately could observe all of the three reproductive organs in their specimens. The roof of a sporangial conceptacle is perforated by 25–30 pores in accordance with the description given by Mason[4]) as clearly shown in Fig. 4, a photomicrograph taken from the upper surface under lateral illumination.



(1), (2) habit of plants covering articulations of *Pachyarthron cretaceum* from Muroran; (3) vertical section through thallus base penetrating into host tissue; (4) surface view of thallus showing pores perforating roof of sporangial conceptacle; (1) ×1; (2) ×1.5; (3) ×30; (4) ×45.

References

1) Tokida, J., and Masaki, T., Bull. Fac. Fish., Hokkaido Univ. **10**: 83 (1959). 2) Masaki, T., and Tokida, J., ibid. **10**: 285 (1960). 3) ——, and ——, ibid. **11**: 37 (1960). 4) Mason, L. R., Univ. Calif. Publ. Bot. **26**: 313 (1953).

* Phycological Laboratory, Faculty of Fisheries, Hokkaido University, Hakodate, Japan.
北海道大学水産学部水産植物学教室

Bot. Mag. Tokyo **73**: 498 (December 25, 1960)

雑　　録

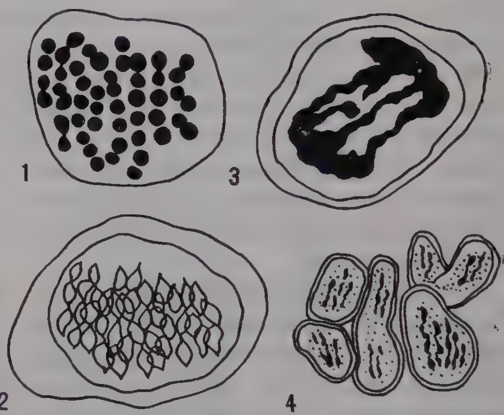
ドクダミの減数分裂

三　原　　勉*

Tutomu MIHARA*: On the Reduction Division of *Houttuynia cordata* Thunb.

1960 年 4 月 27 日受付

ドクダミ (*Houttuynia cordata*) の細胞学的研究は筆者の知るかぎりでは最初に Shibata and Miyake (1908)[1] が胚のうの形成を観察して，この植物には減数分裂をするものとそうでないものがあり，減数分裂をしないものが単性的に胚形成をするようであると報告した．その後に Okabe (1934)[2] が上記の結果をさらに確認し，染色体数が約 $2n=96$ であるとした．筆者は本植物の花粉母細胞の減数分裂を観察してつぎのような結果を得たので報告する．材料のドクダミは愛媛県松山市に自生するものを採集し葯をスライドガラスの上で押しつぶし aceto-carmine 液で固定し染色して観察した．減数第一分裂の metaphase で非常に親和状態の強い丸い形をした 48 個の 2 価染色体を観察したが，これらの 2 価染色体中で 2 個または 3 個が 2 次接合しているものが認められた (Fig. 1 および 2)．anaphase では chromosome bridge が観察されたが (Fig. 3)，その状態は高次倍数体植物の減数分裂にみられるように 1 細胞中に 2～4 本も形成されているのが認められた．



Figs. 1–4. Meiosis in P.M.C. of *Houttuynia cordata*.
1. Chromosomes at MI (polar view).
2. Chromosomes at the same stage (side view).
3. Chromatid bridges at AI.
4. An abnormal tetrad. (× 640)

減数第二分裂の結果形成される 4 分子には核の大小と細胞質量の差異のいちじるしい奇形の花粉細胞を多数観察した(Fig. 4)．以上観察した事柄のうち減数第一分裂の metaphase で 2 価染色体の 2 個または 3 個が次接合していること，および chromatid bridge の状態から，この植物が染色体の倍加によって倍数体を形成しさらに長年月の間に遺伝子陶汰をしてきて構造雑種になったものと考えられる．このような減数分裂の異常がこの植物の受精不能を招き前研究者らの研究にある $2n$ の胚が単独発育をするようになったのであろう．

文　　献

1) Shibata, K., and Miyake, K., Bot. Mag. Tokyo **22**: 281 (1908). 2) Okabe, S., ibid. **48**: 7 (1934).

Summary

The somatic chromosome number of *Houttuynia cordata* Thunb. is 96. At the first metaphase in P.M.C., 48 bivalents tightly paired are observed. Some bivalents show secondary association. In a few cells, chromatid bridges are seen at anaphase. Abnormal tetrads with different number and shape of microsporocytes are often observed.

* Kamogawa middle school, Matsuyama, Ehime, Japan. 愛媛県松山市立鴨川中学校

本 会 記 事

第 25 回 (大 阪) 大 会

第 25 回大会は，11 月2日（水）から4日（金）までの3日間，大阪大学医学部，理学部を会場に開催された。今度の大会は，約400名の正会員と，約100名の臨時会員が参加して，これまでに例を見ない盛会となった。しかし，3日の夜おこなわれた藻類学会の席上，本会の評議員である瀬川宗吉氏がたおれられ，4日未明逝去されるという不幸な事件があった.

評議員会（11 月1日午後5時 30 分，大阪大学理学部会議室）

出席者： 評議員 22 名（欠席4名)，会長，幹事長，幹事3名　　　　計 27 名

議題： 1. 役員移動. 2. 会長，評議員改選. 3. 会員移動. 4. 34 年度決算報告. 5. 35 年度会計中間報告. 6. 植物学雑誌刊行経過および予定. 7. 図書の交換，寄贈の状況. 8. 会費値上げ.

明年3月におこなわれる会長選挙にさきだち会長候補者として芦田譲治・服部静夫・和田文吾の三氏（アイウエオ順）を選出し，推薦することに決定した.

また文部省学術奨励審議会科学研究費等分科審議会委員候補として，原寛，林孝三，門司正三の三氏を選んだ.

明年度予算について会費900円案と1,200円案が提出され，会費値上げの理由として，幹事長よりつぎのような説明がおこなわれた。

(1) 論文の掲載を早くするために，雑誌のページ数を増す必要のあること.（900 円案では 460 頁，1,200円案では 560頁） (2) そのためには編集幹事を増す必要のあること. (3) 雑誌が厚くなることによつて関係経費（郵送費，印刷費など）が増大すること。(4) 文部省からの刊行助成金が年々減少してゆくこと. (5) 諸物価の値上がり.

現行の会費では，投稿論文数などよりみて会の健全な運営は困難であると思えるので年額1,200円，終身会費 20,000 円に値上げする方がよい.

この説明に対して，活発な論議がかわされたが，結局出席した評議員全員が値上げの止むを得ないことを了承して，3日の総会にかけることになった.なお植物学雑誌の論文のうち，和文のものの割合を増してもらいたいという要望がだされたが，これに対して，欧文の論文を別扱いにしていないが，投稿が欧文にかたよっていることによるためであり，また会員からの抄録，雑録の投稿を歓迎していることがのべられた. また名誉会員，特別会員の推薦が話題となったが，資料がそろっていないので，来年の大会で改めて検討することになった。

総　会（11 月3日午後1時，大阪大学医学部講堂）

会長の挨拶ののち，幹事長よりつぎの諸事項の報告があり，総会出席者の承認を求めた.

(1) 役員: 移動の報告および承認（植物学雑誌本年4月号に掲載）

(2) 現在会員の状況報告（昭和35年10月 10 日現在）会員総数 1346 名. うち名誉会員 19 名，特別会員 22 名，外国通信会員7名，終身会員 50 名，通常会員 1248 名.

(3) 会員移動の報告（昭和 34 年9月1日—昭和 35 年 10 月 10 日）新入会 144 名，死亡4名，退会 19 名，除名 34 名，差引増加 88 名.

(4) 植物学雑誌刊行経過および予定の報告.（別表）

(5) 図書の交換，寄贈の状況報告（交換：国外受理 96，国外発送 82，国内受理 34，国内発送 33，寄贈：国外受理 11，国外発送 2，国内受理 76，国内発送 6，予約購読 276)

(6) 昭和 34 年度決算の報告（昭和 34 年1月—昭和 34 年 12 月，植物学雑誌本年3月号掲載).

(7) 来年度大会に関する報告（昭和 36 年 10 月中旬，東京大学理学部で開催，なお 37 年度は名古屋大学の予定）

(8) 会費値上げ，ならびにそれにともなう会則の一部変更についての承認.

評議員会での討議の模様が幹事長から報告され，出席者の討議を求めた. そして採決に入り，出席者 128 名のうち117 名の賛成を得て，会費の値上げは承認された。したがって会則，付則第1　会費の第1条を次のように変更することが承認された. なお

新通常会費は昭和 36 年度から実施される．

第1条　通常会員の会費は年1,200円とし 400円ずつ分納することもできる．終身会費は20,000円とする．

このほか国外在住会員に限り植物学雑誌の送料を負担する．

最後に会長から今回の大会の運営にあたられた大阪大学，大阪市立大学，神戸大学の方々に，感謝の辞を述べ，また大会会長三木茂氏から大会参加者に挨拶があって，総会を終った．

植物学雑誌刊行状況

	論文数	ページ数
1960 年度（小倉記念号）	97	602
1957 年度（75 周年）	77	438
1958 年度	63	448
1959 年度	65	488
1960 年度 1 月号	5	36
2 月号	7	44
3 月号	7	44
4 月号	6	40
5 月号	7	40
6 月号	12	64
7—8 月号	10	68
9 月号	8	42
10月号	11	68
11—12月号	11	62
	84	508

通　常　講　演

分類・地理・形態

川戸峰子・信夫隆治：放線菌の窒素源利用について．I．NO_3 および NO_2 について

増田染一郎：Myxobacteria の水中子実体について

小林艶子：南極産ケイソウ *Navicula muticopsis* V. Heurk の変異

福島　博：南氷洋の着色氷の生物学的研究

根来健一郎：南氷洋の浮氷を彩どる藻類

熊野　茂・瀬戸良三・広瀬弘幸：カワモズク科数種の嚢果形成過程の比較

瀬戸良三・熊野　茂・広瀬弘幸：再びカワモズク属の Chantransia stage について

丸山　晃：北海道東北海岸湖沼のコッコイド・ランソウ相

猪野俊平・熊谷信孝・石井慶三：アミジグサ目の形態発生．I，II．アミジグサ目数種の四分胞子嚢形成と成熟分裂

西林長朗・猪野俊平：コンブ目の形態発生学的研究．IV，V，VI．二三コンブ目植物の遊走子嚢発生と遊走子形成

桃谷好英：蛋白質から見たカエデ属の類縁について

藤田安二：ナギナタコウジュ，タイワンナギナタコウジュおよびフトボナギナタコウジュ

三木　茂・粉川昭平：九州の遺体植物

堀川芳雄：本邦における熱帯性植物の北上分布

堀川芳雄・関　太郎：*Brotherella henoni* (Duby) Fl. カガミゴケについて

高木典雄：中部高山地域における Dicranaceae（シッポゴケ科）蘚類の分布

井上　浩：ゼニゴケ目植物の胞子発芽，特に第一次仮根形成の型

椿　啓介：南極採集品から分離した糸状菌について

曽根田正己：南極土壌より分離せる酵母菌について

信夫隆治・川戸峯子：青緑水溶性色素を生産する放線菌の一新種 *Streptomyces indigoferus* について

寺川博典：高等菌類の分離と再生

佐藤正己：螢光分析法によるムシゴケ属地衣の分類

板垣史郎：*Micrococcus glutamicus* の細胞学的研究（第5報）．有機酸の形態におよぼす影響 —主として branching について

村岡節雄・野口　彰：ヨツバゴケ類の無性芽形成と発芽

髙橋千裕：シダ配偶体の暗培養

小野記彦・榎本雅敏：ミジンコウキクサにみられる老化とその回復現象

岩崎尚彦：シャジクモ科植物の生長点の分化と器官形成．IV．*Chara Braunii*.

吉田　治：フウトウカズラの胚のう形成についての二・三の考察

及川公平：アマナの胚嚢について

福本日陽：*Bryophyllum* の不定芽形成について

原　　襄：オニシバリの生長点構造

熊沢正夫：ハマオモト属における分枝法

上野実朗：裸子植物花粉の形態

原田賢之・村上道夫・竹岡政治：禾本科植物花粉の表面微細構造

幾瀬マサ：蜜蜂の花粉だんご (Pollen loads) の検定

加藤一男・渡辺光太郎：イネ科以外の他科植物における柱頭反応

塙　順：葉の脊腹性についての一実験

秋山　優：本邦産土表性藻類 ***Fritschiella*** の生態

野田光蔵：佐渡海峡の海藻

新崎盛敏・徳田　広：東京湾産ヒトエグサの生活史，特に ***Gomotia*** 型発芽体について

長谷川由雄：ミツイシコンブの生態学的研究．II．生活史について

田中　剛・野沢洽治：南西諸島産ウミウチワ属について

豊国秀夫：ユウバリリンドウの群

小山鐵夫：サルトリイバラ属の分類

鈴木昌友：東北地方南部のカシワバハグマ属植物

原田市太郎：セキショウモ属の核型

浜田秀男：印度支那稲の分類と分布地域

細胞・遺伝

佐々木正人：車軸藻類における染色体数

益森静生：***Artemisia*** 属数種における細胞学的研究

荒野久雄：邦産キク亜科における核型，特に一属一種の種について

佐藤重平：　ショウガ目植物の原始核型と安定核型

神野太郎：***Rubus Nishimuranus*** Koidz. の子孫の細胞遺伝学的研究

西岡泰三：ニガナ類植物の分化についての2・3の知見

浅野　明：海岸植物の核学的研究，第4報

辰野誠次：ケゼニゴケの倍数性とその本邦ならびに近域における分布について

稲荷山資生・竹村英一：ヒガンバナ属の人工雑種について（第4報）

増淵法之：小麦における枝穂の遺伝学的研究

福田一郎：オオバナノエンレイソウの自然集団に現われた染色体異常

木村劼二・藤生みさ子：ウシグソヒトヨの不開傘子実体

竹中　要：進化の一例証

佐藤進一：ムラサキツユクサ倍数種の DNA (Feulgen) 含有量について

松浦　一・岩淵雅樹：細胞分裂におよぼす塩類の影響．I．NaCl 処理による ***Tradescantia*** PMC の分裂異常

三木寿子：キカノコユリの子房内における物質交代

山岸秀夫：螢光染色法による細胞内微小カ粒の分類

伊倉伊三美：シダ類精子の生存時間や形態におよぼす精子毒の作用

土井田幸郎：タデ属植物の発生学的研究．IV．タデ属数種の花芽形成および花粉粒形成におよぼす生長物質の効果

川松重信：アカウキクサ (***Azolla imbricata*** Nakai) の根の電子顕微鏡的観察

左貝アイ子：植物細胞のオスミウム固定についての電子顕微鏡的研究．III．pH の影響について

重永道夫：花粉母細胞で得られた電顕像について

神谷　平：接合藻類の電子顕微鏡観察

丸山圭蔵：葉緑素欠損植物の葉緑体の電子顕微鏡的研究

新家浪雄：***Volvox*** 細胞の電子顕微鏡的構造

松浦　一・谷藤茂行・岩淵雅樹：X線照射による染色体異常頻度におよぼす二・三化学物質の影響

松浦　一：染色体基質の構造について

湯浅　明：コウボキンの紡錘体について

島村　環・太田敬久：核分裂機構の映画による解析

吉田吉男：二三の細胞質活性におよぼす核の相関性

武久　慎：ソラマメの体細胞染色体におよぼす EDTA 処理の効果

石田政弘：***Marchantia polymorpha*** の核酸について

横村英一：***Vicia faba*** の根端細胞核の DNA 量について

下斗米直昌・進藤公夫・田羅征伸：天然属間雑種ノコンギク×オオユウガギクの細胞学的研究

大野林二郎：トウガラシとシシトウガラシの正逆接木にみられる種々なる変化について

木原　均・末本雛子：一粒系コムギにおける左右性の発現機構について

木原　均：左右性の決定に関する考察

生 理・生 化

増田芳雄：オーキシン作用による細胞壁ゆるみとカルシウムの関与
依田静子・芦田譲治：IAA の浸透価におよぼす影響
猪狩盛夫・長尾昌之：根におけるインドール酢酸の吸収ならびに生体内変化
長尾昌之・斎藤周夫・笹岡　信：シュウカイドウ種子の発芽におけるジベレリンと光の作用
和田清美：小麦，大根の無菌培養におけるジベレリンおよびインドール酢酸の効果について
村上　浩：種子の登熟および発芽過程におけるジベレリンの消長
勝見允行：黄化エンドウ茎切片に対するカイネチンの影響 II
山田妙子・橋本　徹・師尾武子・高橋憲子・八巻敏雄：タバコ種子の GA による発芽におよぼす無機塩類の影響
師尾武子・橋本　徹・山田妙子・高橋憲子・八巻敏雄：タバコ種子の GA による発芽におよぼす有機酸塩類の影響
橋本　徹・師尾武子・山田妙子・高橋憲子・八巻敏雄：タバコ種子の発芽におよぼす燐酸イオンの影響
瀕野悍二・芦田譲治：酵母の銅訓養中における遺伝的変化（II）
村山徹郎・芦田譲治：銅耐性酵母の有機酸およびアミノ酸代謝　III
宮本典子・芦田譲治：酵母菌物質代謝の銅阻害
中村　運：酵母のカドミウム耐性における核酸および蛋白質代謝の意義
森下日出旗・奥田正男・高田英夫：ポリエチレングリコール高張環境におけるコーボの生理
山本　武・高田英夫：ストロンチューム高張環境によるコーボ裸プロトプラストの形成と再生
柳島直彦：酵母の呼吸系欠損変異とオーキシン
永井　進：コーボの不安定な菌株について
西上一義：酵母の呼吸の比較生理 glucose, gluconate, pyruvate, arabinose, ethyl alcohol の酸化について
奥田慎一：野生酵母 *Hansenula saturnus* の胞子形成
倉石　衍：酵母のパントテン酸欠乏による死細胞出現
柴岡弘郎・八巻敏雄：ヒマワリ属の葉に含まれる生長阻害物質
林　克己：トウキビの芽生えにおいてアミノ酸の組成におよぼす生長ホルモンの影響
小西通夫：ナフテン酸の根の生長促進作用について
萩本　宏・小西通夫：担子菌子実体の生長を促進する作用物質の研究．V．ツクリタケ（西洋マツタケ）子実体の生長ホルモンとしてのアミノ酸
丸重啓二・丸重靖子：アサガオの芽生における日長感受性の成立と代謝
片山忠夫：稲属各種の感光性の研究
藤伊　正・石川茂雄：種子の発芽における短日的光週性
中山　包・白木健助：サボンソウの種子発芽に対する温度と光の影響
河原　晨：ヒシモドキの発芽について
巖佐耕三・生物研究会：花粉の発芽に対する無機物，特に硼素の発芽に対する効果
市村国彦・井沢三生・太田行人：発芽種子の可溶性 RNA
堀　武義：マツバボタンの開花閉花に伴なう各器官における生長素の消長
堀江格郎：ムラサキツユクサの花の開閉に関係する要因
加藤勇夫：気孔発生過程の表皮組織におけるフォスフォリラーゼ作用
相馬悌介：気孔の開閉運動に対する通気の影響について
山田晃弘：トウゴマ発芽時におけるグリセリン代謝と脂肪酸代謝との関係
辻　英夫・浜田秀男：イネおよびエンバクの発芽に伴なう芽生中の酸溶性燐酸エステル量（特にイネの $\varDelta 7P$ 量）の変化
石川　鉱：秋まきコムギ胚の低温処理中における物質代謝系の変動，特に核酸の代謝について
杉村康知・薬師寺英次郎：数種の海藻のチトクロームについて
今関英雅・山本時彦・瓜谷郁三：オオカメノキの葉の二糖配糖体加水分解酵素
安田　齊：白バラ花弁に対するロイコアントチアン反応

遠藤　徹：パンジーその他の青色花および紫色花のアントシアニン

林　孝三・斎藤規夫・三井清司：ヤグルマギク青色花のメタロアントシアニンについて

桑名　誉：アカパンカビの高温感受性致死突然変異株の遺伝生化学的研究

佐々木喜美子：ベゴニヤの酸化能に関する二・三の知見

笠巻明子・佐々木昭治・宇佐美正一郎： *Proteus vulgaris* による嫌気的条件におけるコハク酸生成機作について

和気和民・宇佐美正一郎：クロカビの分生胞子形成期におけるいくつかの知見

鈴木　昇・奥田　聰・鈴木　旺：*Azotobacter* の窒素代謝

井上行雄・久保秀雄： *Streptomyces griseus* の物質代謝．IX．Intact mycelium によるアミノ酸酸化についての研究．Part 1．培養各期のアミノ酸酸化の概要

井上行雄・久保秀雄：Part 2．Intact mycelium によるモノアミノモノカルボン酸の酸化

上坪英治‥カナダモの原形質流動におよぼす光の影響

山段　忠：フラスモ細胞におよぼすキレート剤の影響

田沢　仁：湿室中に置いたフラスモの浸透価におよぼす光の影響

永井怜子・田沢　仁：フラスモの浸透調節における膜電位の役割

小田健二：ジャジクモの膜電位とイオン分布

柴岡孝雄：微小電極法によるオジギソウ活動電位の研究

須田省三：オジギソウの抗興奮性物質について

鳥山英雄：オジギソウの細胞生理学的研究(第12報)ー紐状装置の運動現象

鳥山英雄：オジギソウの細胞生理学的研究(第13報)ー昼夜によつて形態的変化を示す小体について

遠藤沖吉：光遮断効果の Latency について

松下亀久：TMV の増殖に関する研究

牧野利一：サルモネラ菌によるクエン酸の代謝

香山時彦：細菌多糖類 K.C.G. の制ガン作用（第1報）

伊藤太郎：アカパンカビの誘引性ホルモン様物質の分離

大槻虎男：好稠糸状菌の研究

西尾隆昌：抗酸菌の呼吸におよぼす滲透圧の影響について

賀来章輔‥植物組織の凍結曲線の分析（III）

畠山伊佐男：緑葉の生・死組織の氷点の差異ー生体膠質結合水の示唆

畠山伊佐男・河野　清：吸水力におよぼす外液の界面張力の影響

稲葉耕三：植物の窒素代謝におよぼすカリウムの影響．（II）．RNA への P^{32} の組み入れについて

本田　稔：水分欠乏植物の光合成速度と呼吸量について

高沖　武：水分欠乏植物における2・3の酵素活性について（第2報）

相見霊三：高等植物（イネ）体内における酸素の積極的輸送機能について

山本昌木：*Phytophthora infestans* (Mont.) DeBary 系統の菌体成分と病原性について

岡本　尚：好塩性クラミドモーナスの細胞構造に対する種々の溶質の作用

藤井良平：ウキクサの休眠体の形成

菅井道三：シダ配偶体の造精器形成因子について

渡会彰彦：葉緑体成分分子比と光合成単位

西田晃二郎：光照射下に葉から排出される $C^{14}O_2$ について

藤茂　宏・佐藤公行：緑色植物の光化学的亜硝酸還元系の生化学的研究（その3）

桜井英博：キャベツ白葉の緑化に伴なう光合成に関連した機能および物質の発達について

伊沢清吉：葉緑体におけるビタミン K_3（メナジオン）の酸化還元とヒル反応

加藤　栄：クロレラより抽出した二三の酸化還元蛋白について

千葉保胤・菅原　淳・佐々木　弘：アルミナ処理をした葉緑体の photophosphorylation

菅原　淳：葉緑体への P^{32} のとりこみ．II．各分画の specific activity

宮地重遠：クロレラの細胞内における核酸および燐蛋白質への燐酸導入機作に関する研究

服部明彦・藤田善彦：フィコビリン暗生成過程における細胞内物質の変動

西村公臣：ゴフンゴケから分離された藻の培養

尾形英二：コンコセリス糸状体の生長と炭酸源について

入来義彦・三輪知雄：緑藻細胞膜間粘質物の生化学的研究．I．ヒトエグサ（*Monostroma*）の細胞膜間質

福田育二郎・児玉公一：藍藻細胞の同調培養による生理学的研究．その1．窒素代謝について

生　　態

南川　幸：鈴鹿山脈中北部の植物群落

横川広美：山陰地方のアカマツ林について

秋武和俊・鴫川　誠・細川隆英：桜島の植生構造．I．植生一般および熔岩について

小村　精・宮田逸夫：桜島の植生構造．II．類似度からみた各熔岩上の植生

田川日出夫・小谷信矢：桜島の植生構造．III．熔岩上の植物の分布パタンと群集の分岐度

野本宣夫・佐伯敏郎・門司正三：高等植物の葉の光・同化曲線

楠元　司：沿海地ならびに高地の常緑広葉樹の光合成および呼吸能力

佐伯敏郎：葉の生長におよぼす物質生産の影響

高田和男・黒岩澄雄・広井敏男・萩元育夫：異なった相対照度下における生長の解析

高田和男・黒岩澄雄・岩城英夫：巣まき植物の生長におよぼす巣内個体密度の影響

鈴木時夫：モミーシキミ群集について

矢野悟道：植物地下器官の生態学的研究：砂丘植物について

笠原安夫：耕地雑草群落の構造（I）

森　千春：雑草遷移に関する考察（3）

飯泉　茂・菅原亀悦：ウマタテバにおける再植生過程

辻井達一・小島　覚：鹿部放牧地のドクゼリ集落

菅沼孝之・井上敬子・小清水卓二：奈良若草山の植物群落．3．ススキートダシバ群落の季節的遷移について

岩城英夫・翠川文次郎：霧ヶ峯草原における各種群落の分布について

二村坦孝：湖沼における二・三の水生不完全菌類の分布の特性について

鈴木静夫：湖底泥における水生菌類の分布と生態

今堀宏三・須賀瑛文：湖沼における Charetum の遷移

坂本　充・西条八束：冬期湖沼における植物プランクトンの物質生産について

市村俊英・有賀祐勝：海洋植物プランクトンの光合成特性と基礎生産における意義

宝月欣二・岡西良治・菅原久枝：植物性プランクトンの生長におよぼす水草の影響

塚田松雄：後氷期の花粉分析的研究．V．最終晩氷期以後の花粉分布図

倉内一二：伊勢湾台風の害と回復状況――塩風害と海岸林II

高橋基生：春化処理の根系呼吸におよぼす影響

高橋基生：特異分布ならびに生育に対する生態学的研究．第1報，御蔵島における寒地植物と暖地植物の共存現象

堀川芳雄・岡本　香：広島県産スゲ属植物について

鈴木貞雄：チマキザサ類 *Sasa* sect. *Eusasa* の葉の隅どりの生態

鴫川　誠・秋武和俊：ヤドリギ類の宿主選択について――おもに植付実験について

吉良竜夫・庵原　遜：若いクロマツ人工林の蒸発散量

シンポジウム

話題 I　植物の系統

11月4日　13.00～16.00

第25回大会準備委員会，特に広瀬弘幸氏がこのシンポジウムを企画し，準備し，話題提供者，司会者を選び，会場を管理された．話題提供者はつぎのとおり．

加崎英男氏（都立大・理・生物）：生殖器管の形質からみた見解．

植田利喜造氏（教育大・理・植物）：生理学的形質からみた見解．

三木　茂氏（大阪市大・理・生物）：古生物学上の二，三の例証．

第2番目に予定されていた瀬川宗吉氏（九大・農・水産）：生活環の形式からみた見解　は同氏が4日早朝死去されたため，中止となった．参加者全員で故瀬川宗吉氏に対し黙祷をささげてシンポジウムを閉じた．

加崎氏はたとえば門 phylum のような大きな分

類単位の系統を考えるとき仮説的な先祖群を考えるわけであるが，そのためにどうしても広い生物分類体系の確立が必要となる．それで Smith の植物体の型の進化をとりさらに Sporne (Am. Journ. Bot **46** : 385) の考えを紹介しそれを考えあわせて，運動性あり，色素をもった単細胞の先祖を考えて，これから *Volvox* 式にあつまったもの，パルメラ状に群体をなすもの，胞子状になったもの，アメーバ状になったもの，非細胞性になったものなどの先祖型がでて，それらがそれぞれの傾向をふかめてゆき，嚢胚動物，一般植物，管状植物，粘菌体植物，非体腔動物に進化していくことが考えられるとした．ついで主として藻類における生殖細胞，生殖器管，生殖法の分化の系統的関係を多くのスライドで図示した．

植田氏は植物の系統が形態学の方向からのみされるのではなく，形のもとはそれをつくっている物質によるのであり，植物の生理またはその結果である生産物から，よくその親疎関係，したがつて系統を推察でき，従来の形態発生学的系統学に対し生理生化学的系統学の分野が今後，発達すべきことを最近の研究を綜合して多くの例をのべられた（蛋白質沈降反応，遊離アミノ酸，核酸組成，葉緑体内クロロフイル含量，細胞膜成分，色素など）．

三木氏は古生物が系統を考えるうえにおいて大きな役割を果した例を，ソテツシダ類，*Rhynia* の例で示した後，狭い範囲ではあるがと前置きして，*Hemitrapa* などの例 (Proc. J. Acad. **35**: 6 参照) につき具体的にのべられた．*Hemitrapa* は *Trapa*（ヒシ属）および *Lythrum*（ミソハギ属）の古い型とくらべると，その中間形であること，*Eoeuryale* は種子のみであるが明らかに *Euryale*（オニバス）とは異なること，ヒルムシロ属，イバラモ属との関係など説明した．

瀬川氏の生活史，広い意味での植物の発生がのべられなかったのは残念であるが，氏は生活環のよび名の統一をもとめ，それらの生活史の型がどのように各群で出現しているか，その系統との関連はどうかを述べられるはずであった．不幸にも氏の話題提供のないため系統を考える場合に形質と環境のかねあいがじゅうぶんに論議されなかったのは残念である．

一般討論にうつり多くの人の熱心な活発な討論があって休むひまもなく，ついに時間がきて討論もうちきらざるを得なくなった．ここに論者の主旨を紹介することはできないが，最も多くの討論が系統を明らかにするために形態的特徴か生理生化的特徴か，その関連はどうかという点にあった．話題が大きいので充分話しあい結論をだすに至らなかったが司会者としてつぎのような点を特に感じた．

（1） 大きな群（門，綱など）の系統と小さな群（属，種，変種など）の系統を一応分けて話しあった方がよい．（2） 体系にしろ系統にしろ一つの形質によるものと多くの形質の総合によるものとがあり，比較する場合にそれを意識しなければならない，生理生化的特徴による系統が，形態的特徴による学説の考えと矛盾するとき，このことは特に注意されなければならない．（3） 形質が簡単から複雑へとみて系統を考えるが複雑から簡単への場合もあり得るので内外の環境要素の重要性を改めて考えなおす必要がある．（4） つきつめていくと形と働きの相互関係という歴史の古い問題につきあたる．（5） 発生学的方法によって形態学は進歩した．生理学生化学から系統を考えるうえに最終生産物が最も問題とされているがやはりこの過程の型が重要ではないか，生理の相同相似も研究されるべきである．（6） Mez (1914) の蛋白質による系統などの研究が大きくうかんだことがあったが不完全とされていた．現在のすすんだ生理学，生化学によってこの方向からの体系と系統の問題が進歩してきたことを喜び今後の発達を期待する．

（シンポジウム司会者　木村陽二郎記）

話題 II　細胞の増殖と分化

11 月 3 日　14.30～17.30

司会　小清水卓二

II·1　高尾昭夫：胚の発生と物質の貯蔵過程（座長　島村　環）

高尾氏は，クロマツと二，三のマメ科植物を用いて，組織化学的方法で多糖類とタンパク質の消長をしらべた結果をのべた．胚が分化生長するにつれて，種々の物質が貯蔵されるが，それらの物質は組織の相同のいかんにかかわらずほとんど同様である．胚発生のさい，まわりの組織を通して養分の供給をうけるから，胚座，子房，胚珠の管束の走りか

た，胚柄，胚をとりまく液状物質のはたらきをしらべる必要がある．種皮は，それを作る細胞壁が厚くなるにつれて不透過性状態となり，これが種子の休眠と関係がある．ゆえに，今後の胚発生の研究は，形態的方法だけでなく，生化学的方法や胚培養法で追及すべきであることが指摘された．この講演に対して，及川公平氏から，胚組織の細胞質は貯蔵とは無関係によく色素で染まるが，この点の検討はどうかという質問があり，演者から，その点は十分考慮しているが，胚珠心の場合にはあまりそれを考慮する必要はないと思う，との答があった．

II·2 中沢信午：分化における皮部細胞質の役割（座長 新家浪雄）

この講演では，藻類の卵およびシダ類の胞子においては，遠心力に安定な有極性構造が粘性の高い皮細胞質のうちに存在する，という考えがのべられた．この有極性分子の整頓配列は，細胞の主軸のまわりにラセンをえがいていて，それが形態形成物質の部域的配分に役割を演じ，部域的に特異な細胞分化をおこし，ついで部域特異のパターンを形成すると考えられる．水野忠款氏から，皮質細胞質の分化は電子顕微鏡でみとめられるか，との質問があり，座長の指名によって植田勝己氏がつぎのように答えた．ユーグレナの細胞皮膜には，溝がラセン状にとりまいているが，この溝にそってミトコンドリアや葉緑体がならんでいるのがみられる．しかし，電顕分解能では，皮質には分化がおこっているとは思えない．ついで植田氏は，皮質の分化が直接に証明できないとしたら，細胞膜の局所における質的ないし量的な差異を考えるだけで説明ができはしないか，とただした．これに対して演者は，どこまでも細胞質皮質を重視している．これは動物発生でも皮質が重要な役割をしているのと同様で，細胞膜の厚薄は結局細胞質のはたらきによってできたにすぎないと考える，と答えた．

なお，形態形成物質とRNAの関係（吉田吉男氏），皮部細胞質と原形質膜をふくむうすい皮層と考えてはどうか(木村陽二郎氏)などの質問があり，それぞれ同感であるが，具体的な検証は今後にまちたい，との演者からの答があった．

III·3 森本 孝：ユーグレナの呼吸阻害と細胞分裂（座長 湯浅 明）

森本氏は，二分法のみで増殖するユーグレナを材料として，チトクローム系をはじめとする末端酵素阻害剤，TCA cycle 系阻害剤，解糖系阻害剤，酸化的燐酸化系阻害剤の代表的なもの約 30 種をもちい，呼吸および分裂に対する影響をのべ，他の動植物を材料とした結果と比較した．

形態形成を，形態学と生理学との協力によって追求する分野は，きわめて重要かつ興味ある問題で，形態学・分類学・生理学などの各分野から約150名におよぶ参会者を集め，活発な質疑応答のうちに終った．

話題 III 日本における遷移

11 月3日 14.40〜17.40

III·1 手塚泰彦：遷移の機構—土壌要因の役割を中心に（座長 堀川芳雄）

手塚氏は，火山島伊豆大島での一次遷移の機構を，群落の土壌に対する反作用に基礎をおいてのべた．裸地→荒原→低木林→常緑・落葉混合林→照葉樹林と遷移が進むにつれて，これらの群落の種類密度・組成・葉量・現存量がどのように変化するかが明らかにされたが，葉面積指数は 10 前後に，生産力は 60 ton/ha·yr に収斂する傾向がみとめられた．N, P, K などの主要な元素の年廻転率は，それぞれ 4.3〜3.4，2.9〜2.4，0.3〜0.7 程度で，低木林期以後はあまり大きな差異がない．以上のような諸量についてもっとも変化のいちじるしいのは，荒原から低木林への遷移の途中である．

III·2 沼田 真：二次遷移と遷移診断（座長 門司正三）

沼田氏は，二次遷移の問題点を，a) 遷移の進行にともなって出現する種の特性，b) 土壌中の種子集団，c) 群落の体制化，d) 遷移における動的平衡と長期傾向，の4つに整理し，Salisbury, Knapp など内外の学者のデータを引用してのべた．ついで遷移診断の問題にうつり，現在の状態の判定と，それが今後どういう方向に変化してゆくかを（状態と傾向の診断）客観的に，定量的にはかるためには，群落のいかなる特性に注目すべきかを論じた．とくに，あるシバ型の草原では蘚類指標が状態診断に有効であることや，遷移度（総合優占度・植物の寿命・個体数から計算する）という新しい概念の提唱などが注目された．

III·3 吉岡邦二：わが国火山における一次遷移

（座長　細川隆英）

吉岡氏の講演では，北は北海道駒ケ岳から南は桜島におよぶわが国の主要火山における遷移の実態をしめす，豊富なスライドと表を用いて，長期にわたる観察結果がのべられた．遷移の速度は，火山噴出物の種類によってことなり，泥流，二次火山灰地および浮石地ではかなり速いが，熔岩上ではいちじるしくおそい．とくに前三者では，ふつう常識的に考えられているような，地衣・蘚期→草原期→樹林期の系列がみられず，ただちに草木または樹木（クロマツ，ヤナギ類，カンバ類，ハンノキ類，カラマツなど）がパイオニアとして侵入する．しかし熔岩地では，地衣や蘚類がパイオニアとなる．したがって，ひとつの火山に注目すると，これら堆積母材料の性質に応じて，ちがった遷移系列が平行的に進むことが注意された．

つづいて，上記三座長の司会のもとに，約 70 名の参会者の間で，活発な討論が行なわれた．前半は，鈴木時夫氏の提出した一次遷移と二次遷移の関係について，議論が集中したが，あまりはっきりした結論には達しなかった．術語として一次遷移と二次遷移を区別する必要があるかどうか，ボタ山・洪水あと地などの例で両者をどう判別するか，などがおもな話題となった．

つぎに沼田氏から，遷移の初期に出現する種の特性について若干の補足があり，また現在調査中の竹林でもなかなか公式通りには遷移が進まないことが指摘された．また竹林の遷移にみられる波動的な変化と環境条件との関係についての質問に対して，同氏はむしろ“生活力は変動する”という立場を表明した．また蘚類指標については，堀川氏から若干の疑問点がのべられた．

火山における遷移については，樹木がなぜパイオニアとして登場するかが問題になった．吉岡氏は，コンパクトな草本の根系にくらべて，樹木の根系がルースで，未成熟の土壌中でも，かなりひろい範囲から養分を吸収できる点を指摘し，手塚氏もこれに全面的な賛意を表した．また手塚氏の土壌のサンプリング法について質問があったが，時間不足のため，あまり本質的な討論にはいらなかった．

話題 IV　微生物の代謝

11 月 4 日　13.00—16.00

座長　高宮　篤

IV·1　岩塚　寿・丸山槇子・久野光造・森　健志：硫黄細菌の CO_2 固定

Whole cell をもちいた実験では，イオウの酸化にともなって $^{14}CO_2$ がとりこまれる．イオウを加えなければ，$^{14}CO_2$のとりこみはみられない．CO_2-free air でイオウ酸化をやらせ，N_2 中で $^{14}CO_2$ を加えても固定が行なわれる．すなわち，酸化反応が CO_2-固定に必要な energy を供給するわけである．cellfree preparation をもちいて H_2S の酸化と CO_2- 固定を ADP の存在下で共役させることができる．

IV·2　金井竜二・宮地重遠・高宮　篤：微生物における酸水素反応と共役した炭酸固定

Streptomyces autotrophicus は爆鳴気反応の energy を利用して，CO_2-固定を行なう．CO_2 をのぞいた酸水素気中におき，さらに N_2 中に放置したのち $^{14}CO_2$ にふれさせた場合でも，CO_2-固定がおこる．*Hydrogenomonas facilis* においても，前処理によって CO_2-固定能ができる．

IV·3　尾形昭逸：光合成菌の photosynthesis と chemosynthesis. *Chromatium* sp. の photophosphorylation, pyridine nucleotide reduction と carbon assimilation pathway

Chromatium による photophosphorylation は，酸化物質・還元物質の生成をともなわないビタミン K type cyclic の photophosphorylation であると考えられる．光による reduced pyridine nucleotide の形成はみとめられない．CO_2 の光同化過程で H_2 の消費がみられるが，H_2 による DPN の還元が暗所でおこることがわかった．ATP と $DPNH_2$ の供給があれば，まったく暗所においても CO_2 の同化がおこる．この話題に対しては，cyclic photophosphorylation における電子の流れが実際におこりうるか，ということが問題になった．

（座長　渡辺　篤）

IV·4　森　健志・岩崎秀一・大西智子・鈴木秀穂：細菌の脱窒素反応

脱窒素反応をおこす細菌 *Pseudomonas denitrificans* から，cyt. c^{552} と cryptocyt. c がえられる．cryptocyt. c は NO_2^- と反応するが，電子伝

達における役割は不明である．脱窒素反応に関連する ^{32}P のとりこみが静止菌によってみられ，とりこまれた ^{32}P は nucleotide 類にひろく分布している．酸素存在下に培養した細胞では，脱窒素反応はみられない．またその細胞からは，cryptocyt. c のかわりに cyt. c^{550} がえられる．この話題に関しては，培養条件による呼吸系の変動が討論された．

エクスカーション

はじめ予定した近郊見学二班のうち，六甲山上の高山植物園・森林植物園をたずねるコースが，水害による道路故障のため，中止せざるをえなくなったことは残念であった．

参加者 65 名，バス 2 台，9 時すぎ宿舎太融寺前を出発．大阪市の東北端にあたる茨田町から隣接する大東市にかけて，有名な蓮根の栽培地帯をぬけて，最近開通した阪奈有料道路に入る．七曲りの急坂を生駒山脈の峠までのぼりつめ，さらに南に分かれる登山道路を山頂に向かうところから，曇天の空が晴れあがり秋空がみえはじめた．10 時すぎ着．まだ朝はやく人影もまばらで，晴れのこりの雲のため展望は十分とはいえなかったが，林立するテレビ塔にも紅葉をはじめた木立ちにも，もう晩秋の色が濃かった．山頂で小 1 時間，飛行塔の動きだすのをまちかねて，童心にかえる人もあった．

11 時山頂発．往路をひきかえし，大東市から寝屋川市をへて，北河内郡交野(かたの)町私市(きさいち)につき，徒歩11分で大阪市大附属植物園にはいった．昼食後，園長から園の内容について説明があり，ゆっくりと園内を見学した．ここは，建設に着手してから 10 年目で，約 700 m² の温室をはじめ諸設備がちょうどひとわたり完成したばかりである．かなり大規模な設計と，豊富な温室植物その他のコレクションが注目をひき，終始熱心に見学する人が多かった．2 台のバスは，それぞれ午後 2 時半と 4 時とに私市を出発，大阪駅に直行して解散した．

役員移動

本会庶務幹事は11月より清水碵氏から駒嶺穆氏に交代しました．また 1961 年 1 月より幹事長には，下郡山正巳氏が門司正三氏にかわり就任される予定です．

本会名誉会員斎藤賢道氏は本年 10 月 14 日おなくなりになりました．
本会評議員瀬川宗吉氏は本年 11 月 4 日おなくなりになりました．
ふかく哀悼の意を表します．

日本植物学会